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## MORPHOLOGICAL DIFFERENCES BETWEEN THE "RACES" OF *DROSOPHILA* *PSEUDOOBSCURA*

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### 1. INTRODUCTION

THE two "races" of *Drosophila pseudoobscura*, known as "race A" and "race B," respectively, were first distinguished by Lancefield (1929), who found that on intercrossing they produce sterile male hybrids. In addition to this sterility and other abnormalities of the hybrids, Lancefield further found an incomplete sexual isolation of the races: in mixed cultures intra-racial matings were more common than inter-racial matings.

The only visible difference between the two races detected by Lancefield was that in the shape of the Y chromosome, which was V-shaped in race B and J-shaped in race A. Dobzhansky and Boche (1933) and Dobzhansky (1935a, 1937a) have shown, however, that the shape of the Y chromosome is variable in both races, five distinct forms being present in race A and three in race B. Further, as far as can be determined cytologically, some strains of each race have the same type of Y chromosome. Tan (1935) and Koller (1936) have found the chromosomes of the two races to differ in four inverted sections, two of which lie in the X and one in each of the second and third chromosomes. Again it was shown later that the chromosome structure of the individual races was vari-

able. Certain strains of the two races are identical with respect to gene arrangement in the right limb of the X and in the third chromosomes (Sturtevant and Dobzhansky, 1936; Dobzhansky and Sturtevant, 1938). Only the left limb of the X and the second chromosome may be used as racial differences.

There can be no doubt that the two races represent distinct ecological types. Their geographical distributions are different, and in the regions where they occur together race A occupies warmer and drier habitats than race B (Dobzhansky, 1937b). Poulson (1934) has established that the developmental period of race B is longer than that of race A, the difference being mainly in the length of the pupal stage. The number of eggs deposited by a female of race A is greater than that deposited by one of race B at higher temperatures, while the reverse is true for lower temperatures (Dobzhansky, 1935b). The egg-laying curves of the races differ at all temperatures that have been tried. There is also some reason to believe that the oxygen consumption per unit dry weight differs in the two races (Dobzhansky and Poulson, 1935). Finally, in the absence of food, individuals of race A survive longer than do those of race B (Lilleland, 1938).

In short, the differences between the races are sufficiently profound to remove any hesitation to regard them as species if it were not for the apparent absence of any external morphological differences. The situation confronting us here is not entirely unique in biology, since the existence of types which are physiologically distinct and yet seem morphologically identical is known elsewhere. Such types are often designated by the not too fortunate terms of "biological species" and "biological races." It would seem, however, desirable to have further information about *Drosophila pseudoobscura*, as it is the best studied case of this type.

The genitalia, which often serve to differentiate species of insects, were carefully compared in the two races, but were found to be identical. Professor G. F. Ferris, who

kindly consented to check these observations, reached the same conclusion (private communication). The only hopeful indication of morphological difference found up to the present was obtained in measuring the length of the hind tibiae and counting the teeth of the sex combs in five strains of race A and three of race B (Dobzhansky, 1935c). It remains, however, to be ascertained whether these differences are truly interracial. Each strain consists of the descendants of a single trapped female, fertilized in the wild by one or more males, and kept in the laboratory, in some cases, for many generations. The differences between such strains of the same race could be mistaken, without careful analysis, for genuine racial differences. In order to investigate further this possibility and to establish the racial difference, if it exists, the present work was undertaken.

## 2. MATERIAL AND METHODS

Nineteen strains of race A and twenty of race B were used in the experiments. These strains were obtained from different parts of the distribution area of the species. The strains and their places of origin are given in Table 1.

TABLE 1

RACE A	RACE B
Mara (Shuswap) Lake-3. British Columbia*	Quesnel-5. British Columbia*
Olympic-2. Washington*	150 Mile House-5. British Columbia‡
Crater Lake-3. Oregon*	Pavilion-6. British Columbia‡
Black Hills-5. South Dakota*	Merritt-4. British Columbia*
Pike's Peak-4. Colorado‡*	Campbell River-3. Vancouver Island‡*
Tree Line-3. Colorado‡	Cowichan-6. Vancouver Island‡
Julian E-6. California*	Quinault-23. Washington‡
Sequoia-15. California‡	Quilcene-4. Washington*
Wawona-6. California‡	Seattle-4. Washington‡
Lida-31. California‡	The Dalles-7. Washington‡
Coso-99. California‡	Reedsport-2. Oregon‡*
Awavaz-4. California‡	Sisters-9. Oregon*
Kingston-1. California‡	Crater Lake-2. Oregon‡
Santa Catalina-2. Arizona‡	Lassen-2. California*
Santa Rita-1. Arizona‡*	Lassen-8. California‡
Zuni-8. New Mexico*	Dunsmuir-8. California‡
Georgetown. Texas‡	Wawona-4. California*
Cuernavaca-8. Mexico*	Sequoia-5. California‡
Oaxaca-4. Mexico*	Sequoia-14. California*
	Nojogui-8. California*

\* Wings and tibiae measured and teeth of proximal sex comb counted.

‡ Teeth of proximal and distal sex combs counted.

Approximately 20 (or less) females and an equal number of males, of each strain, were placed in a culture bottle



at 24.5° C., and were transferred to fresh bottles daily. The cultures with eggs in them were allowed to develop at 24.5° C. except where otherwise stated. Cultures that proved to be overpopulated with larvae were then discarded. In the remaining bottles the flies emerging in the first three days were removed and preserved in alcohol. In this way each sample of flies from which measurements were taken comprised individuals from a number of different bottles, though all from the same parents. This technique should on the one hand insure that the flies have developed under good conditions and on the other reduce that accidental variation due to fluctuating environmental conditions in different bottles. The suitability of the method will be discussed later in the light of the experimental results.

### 3. RESULTS

(a) *Sex Combs*. Five characters were studied in the experimental material, three of which, wing length and breadth and tibia length, could be measured on flies of both sexes. The other two, *viz.*, the numbers of teeth in the proximal and distal sex combs of the right front leg, were only to be obtained from males. Two experiments were performed on two different occasions, the proximal sex combs being followed at both times, whereas the wing and leg measurements were only taken on one of the two occasions and the distal sex combs counted in the other set of material. The different strains used in each of the two experiments are marked by the symbols § and \* in Table 1. As four of the strains were followed in both experiments we have twice as many observations on the proximal sex combs of these strains as of the others. The other measurements were followed in but one of the two experiments, and so an equal number of flies from each of the particular strains used were recorded. We may consider the sex comb results first.

As indicated in the introductory section we are concerned with analysis at three levels, variation between individuals of the same strain, between strains of the same

race, and finally between the two races. The most suitable statistical technique for handling a problem of this kind is that of the analysis of variance.

The mean numbers of teeth ( $\bar{x}_p$ ) in the proximal sex combs of each of the thirty-nine strains, together with the sums of squares of deviations of individuals from the strain mean denoted by  $S(x_p^2)$ , are given in Table 2. The results for the four strains used on both occasions comprise counts on fifty male flies, but the remaining strains included but twenty-five males each.

A simple analysis of variance may be performed on the data of Table 2. As there are four strains, each including

TABLE 2  
FREQUENCY OF TEETH IN THE PROXIMAL SEX COMBS

Race A	$\bar{x}_p$	$S(x_p^2)$	Race B	$\bar{x}_p$	$S(x_p^2)$
Santa Catalina-2	6.36	5.76	Quinault-23	6.00	2.00
*Pike's Peak-4	6.00	14.00	150 Mile House-5	5.60	6.00
Kingston-1	5.92	5.84	Pavilion-6	5.64	5.76
Coso-99	6.44	8.16	The Dalles-7	5.76	6.56
*Santa Rita-1	6.22	12.58	Dunsmuir-8	5.96	8.96
Tree Line-3	6.56	8.16	Sequoia-5	5.73	5.04
Awavaz-4	6.64	5.76	Lassen-8	5.64	7.76
Texas	6.68	7.44	Crater Lake-2	5.44	6.16
Lida-31	6.72	9.04	Seattle-6	5.04	2.96
Sequoia-15	6.76	8.56	Cowichan-6	4.56	6.16
Wawona-6	6.72	5.04	*Reedsport-2	5.56	12.32
Olympic-2	6.64	9.76	*Campbell River-3	5.82	9.38
Crater Lake-3	6.76	6.56	Quilcene-4	5.76	4.36
Zuni-8	6.48	10.24	Quesnel-5	5.72	9.04
Julian E-6	6.88	6.64	Sisters-9	6.08	5.84
Cuernavaca-8	5.96	2.96	Lassen-2	5.36	5.76
Oaxaca-4	6.40	8.00	Sequoia-14	5.88	6.64
Black Hills-5	6.80	10.00	Nogoi-8	5.76	10.56
Mara-3	6.36	7.76	Wawona-4	5.92	3.84
			Merritt-4	5.20	6.00
Racial mean	6.4533		Racial mean	5.6273	

All these results are based on counts of 25 males except those marked \* which are based on 50 males.

fifty individuals, and thirty-five, including twenty-five individuals, we have observations on 1,075 flies in all. There is thus a total of 1,074 degrees of freedom in the analysis. As there are thirty-nine strains, 38 degrees of freedom are concerned with racial and strain differences, leaving 1,036 for the variation of individuals round the means of their strains. The 38 degrees of freedom may be further subdivided into 1 for the difference between the two races and 37 for deviations of strains round

their racial means. The arithmetic method of obtaining the sums of squares of deviations of individuals round the strain means, of strains round the racial means, and finally between the races is fully described by Fisher (1936a and 1936b) and need not be discussed here. Having found the requisite sums of squares from the data of Table 2 we obtain the analysis of variance given in Table 3.

TABLE 3  
THE ANALYSIS OF VARIANCE OF NUMBER OF PROXIMAL SEX COMB TEETH

	Sum of squares	Degrees of freedom	Mean square
Races .....	183.2893	1	183.2893
Strains .....	109.1377	37	2.9497
Individuals .....	283.5600	1,036	0.2737
Total .....	575.9870	1,074	

It is clear that there is a significant difference between strains of the same race, as in the absence of such differences the mean squares (or variances) of "strains" should be equal to the mean square of "individuals." The calculation of  $z$  ( $\frac{1}{2}\log_e$  of the ratio of these variances) gives a value of 1.1887, which, for 37 degrees of freedom against 1,036, has a probability considerably less than 1 per cent. of occurring by chance. Hence in testing the evidence for the existence of a difference between the races we must compare the "race" mean square with that for "strains" and not with the value for "individuals," as otherwise we might be led into the error of ascribing to racial difference an apparently significant variance which was really a mere difference between strains. A comparison of the "race" variance with the "strain" variance renders such an error impossible. In making this comparison of the "race" and "strain" mean squares we obtain a  $z$  of 2.0647, or a  $t$  value of 7.883. Either test of significance indicates a very real difference between the races, when entered in the appropriate tables given by Fisher (1936a). Thus the number of teeth in the proximal sex comb provides a genuine external morphological difference between the two races.

The distal sex combs of 25 males from each of 23 strains, 11 of race A and 12 of race B, were also counted, in addition to the proximal comb data. In Table 4 will

TABLE 4  
FREQUENCIES OF TEETH IN THE PROXIMAL AND DISTAL SEX COMBS

	$\bar{x}_p$	$\bar{x}_d$	$S(x_p^2)$	$S(x_d^2)$	$S(x_px_d)$	$\bar{X}$
<b>Race A:</b>						
Santa Catalina-2	6.36	5.24	5.76	4.56	0.84	8.59
Pike's Peak-4	5.92	5.12	5.84	2.64	0.24	8.45
Kingston-1	5.92	5.36	5.84	5.76	1.72	8.57
Coso-99	6.44	5.64	8.16	5.76	2.96	9.23
Santa Rita-1	6.40	5.16	6.00	9.36	5.40	8.95
Tree Line-3	6.56	5.56	8.16	6.16	1.16	9.31
Awavaz-4	6.64	5.36	5.76	5.76	0.24	9.29
Texas	6.68	4.96	7.44	0.96	- 0.32	9.13
Lida-31	6.72	5.48	9.04	6.24	1.36	9.43
Sequoia-15	6.76	5.60	8.56	6.00	1.60	9.53
Wawona-6	6.72	5.08	5.04	1.84	0.56	9.23
Racial mean	6.4655	5.3236				9.10
<b>Race B:</b>						
Quinault-23	6.00	4.88	2.00	2.64	0.00	8.42
150 Mile House-5	5.60	4.64	6.00	7.76	3.40	7.90
Pavilion-6	5.64	4.96	5.76	0.96	- 0.36	8.09
The Dalles-7	5.76	4.80	6.56	6.00	3.80	8.14
Dunsmuir-8	5.96	5.08	8.96	5.84	0.08	8.47
Sequoia-5	5.72	5.04	5.04	0.96	0.28	8.21
Lassen-8	5.64	4.96	7.76	0.96	0.64	8.09
Crater Lake-2	5.44	4.88	6.16	4.64	2.32	7.86
Seattle-6	5.04	4.44	2.96	6.16	0.56	7.24
Cowichan-6	4.56	4.04	6.16	0.96	0.44	6.56
Reedsport-2	5.48	4.20	6.24	4.00	1.60	7.56
Campbell River-3	5.76	4.80	6.56	6.00	1.80	8.14
Racial mean	5.5500	4.7267				7.89

All results are based on 25 males.

be found the mean numbers of teeth of the two combs ( $\bar{x}_p$  and  $\bar{x}_d$ ) together with the sums of squares of deviations of individual observations on each comb round the appropriate strain means [denoted by  $S(x_p^2)$  and  $S(x_d^2)$ ] and also the sums of cross products of deviations of the individual observations [ $S(x_px_d)$ ]. In the case of the four strains used in both experiments, on only this one particular occasion were the distal combs counted and so but 25 males of these strains, as of the others, are recorded in Table 4. In Table 5 are the analyses of variance and co-variance, the last based on the sums of cross products, of the teeth frequencies of the two combs. There is 1 degree of freedom for racial differences, 22-1, i.e., 21, for differences of strains within the races, and 574-22,

TABLE 5

ANALYSES OF VARIANCE AND CO-VARIANCE OF PROXIMAL AND DISTAL COMB TEETH

	Sum of squares	Degrees of freedom	Mean square
Variance of Proximal Teeth:			
Races .....	120.2430	1	120.2430
Strains .....	66.9118	21	3.1863
Individuals .....	145.7600	552	0.2641
Variance of Distal Teeth:			
Races .....	51.1317	1	51.1317
Strains .....	43.8631	21	2.0887
Individuals .....	101.9200	552	0.1846
Co-variance of Proximal and Distal Teeth:			
Races .....	78.4107	1	78.4107
Strains .....	32.3545	21	1.5407
Individuals .....	30.3200	552	0.0551

*i.e.*, 552, for variations between individuals of the same strain. In each case the variance, or co-variance, between strains of the same race is larger than that of individuals of the same strain. The values for the racial differences are also larger than those for the differences between strains of the same race, just as before. Further, since the values for the two combs are not completely correlated ( $r = +0.597$  between strain means) some information presented by each comb is not to be obtained from a comparison based solely on the other one. Thus some composite score, based on a combination of the numbers of teeth in the two combs, will be better than the values of either comb taken separately.

We may proceed to find such a score, or "Discriminant Function," by the method of Fisher (1936c). This consists of taking a linear function  $X$  of the two variables,  $x_p$  (proximal comb teeth) and  $x_d$  (distal comb teeth), such that the difference in score between the two races is maximized with respect to the variability of  $X$  within the races. As we have shown that there exist real differences between the strains of a race we must compute the variability of  $X$  within the races by the use of strain means and not by using individual observations.

We give the two variables,  $x_p$  and  $x_d$ , the coefficients  $\lambda_p$  and  $\lambda_d$  in finding the composite score  $X$ . Thus, an extra suffix denoting A or B,

$$\bar{X}_A = \lambda_p \bar{x}_{pA} + \lambda_d \bar{x}_{dA}$$

and

$$\bar{X}_B = \lambda_p \bar{x}_{pB} + \lambda_d \bar{x}_{dB}$$

giving by subtraction

$$D = \bar{X}_A - \bar{X}_B = \lambda_p d_p + \lambda_d d_d$$

where  $d_p$  is the difference between the race averages of  $x_p$ , and  $d_d$  is similarly  $\bar{x}_{dA} - \bar{x}_{dB}$ .

The theory and calculation of discriminants is fully described by Fisher (*loc. cit.*). We require for this purpose the sums of squares of deviations of the strain means, for both  $x_p$  and  $x_d$ , from the appropriate racial means, and also the sum of cross products of these deviations. These are obtained from the second row of the analyses of Table 5, *i.e.*, the three entries in the "strains" row. We divide these values by 25, however, as the strain means are the averages of 25 observations each, whereas the sums of squares in Table 5 are based on the values of single males.

The values of  $\lambda_p$  and  $\lambda_d$  are given by the solution of the equations

$$\begin{aligned} S(x_p^2)\lambda_p + S(x_px_d)\lambda_d &= d_p \\ S(x_px_d)\lambda_p + S(x_d^2)\lambda_d &= d_d \end{aligned}$$

where  $S(x_p^2)$  is the sum of squares of  $x_p$ ,  $S(x_d^2)$  the sum of squares of  $x_d$ , and  $S(x_px_d)$  is the sum of cross products. Substituting the appropriate arithmetic values, we find

$$\begin{aligned} 2.6765\lambda_p + 1.2942\lambda_d &= 0.9155 \\ 1.2942\lambda_p + 1.7545\lambda_d &= 0.5965 \end{aligned}$$

and so

$$\lambda_p = 0.2760 \text{ and } \lambda_d = 0.1366$$

or putting  $\lambda_p$  equal to 1

$$\bar{X} = \bar{x}_p + 0.4949 \bar{x}_d$$

Then  $D$ , the difference between the average scores of the two races, is

$$0.9155 + (0.4949 \times 0.5969), \text{ i.e., } 1.2109.$$

The significance of the racial difference shown by this score may be tested by analyzing the variance of  $X$  into its two parts, between the races and between strains of the same race. It will be noticed that this test of signifi-

cance is precisely analogous to the test applied to the individual comb values.

It has been shown by Fisher (*loc. cit.*) that the sum of squares between races is given by

$$\frac{n_A n_B}{n_A + n_B} D^2$$

where  $n_A$  and  $n_B$  are the numbers of strains of the two races used in the experiment. In the present case  $n_A$  is 11 and  $n_B$  is 12. Then the sum of squares between races is

$$\frac{132}{23} (1.2109)^2$$

i.e.,

$$8.4147$$

The sum of squares between strains of the same race is found from the formula

$$\lambda_p^2 S(x_p^2) + 2\lambda_p \lambda_d S(x_p x_d) + \lambda_d^2 S(x_d^2)$$

and, as we have put  $\lambda_p = 1$  and  $\lambda_d = 0.4949$ , is

$$2.6765 + [0.9898 \times 1.2942] + [(0.4949)^2 \times 1.7545]$$

or

$$4.3862.$$

There are 23 strains in all and so a total of 22 degrees of freedom. In our previous analyses of the values of each comb taken singly we have subdivided these 22 into 1 between races and 21 between strains of the same race. As we have, however, now emphasized the difference between the races by fitting an adjustable parameter, viz., the ratio of  $\lambda_p$  to  $\lambda_d$ , we must assign 2 degrees of freedom to the racial difference and leave but 20 for the strain variation instead of the original 21. The analysis of variance of  $X$ , obtained in this way, is given in Table 6.

It is desirable to test the increase in accuracy of detection of the racial difference by the  $X$  score over that obtained when either comb is used separately. This can not be done directly, as the  $X$  score has 2 degrees of freedom against 20, whereas the single comb data have 1 against 21. We may, however, achieve this purpose indirectly by

TABLE 6  
ANALYSIS OF VARIANCE OF THE COMPOUND SCORE *X*

	Sum of squares	Degree of freedom*	Mean square
Races .....	8.4147	2	4.2074
Strains .....	4.3862	20	0.2193

\* *z* for the difference between the variances is 1.4771, which for 2 degrees of freedom against 20 is well outside the 1 per cent value of 0.8831. Thus the difference between the races as measured by our discriminant function is large and very significant.

a comparison of the frequency of misclassification. From the mean square, or variance, of strains within races we may calculate, by taking the square root, a standard error to which the strain means are subject. Before doing this, however, we must divide the variances of Table 5 by 25 just as in the calculation of the discriminant, and for the same reason, *viz.*, that the strain means are based on 25 individuals. In the case of the analysis of variance of the *X* score, as given in Table 6, the division by 25 has already been performed.

Thus the mean number of teeth of the proximal comb has a standard error of  $\sqrt{\frac{3.1863}{25}}$  *i.e.*, 0.3596. That of the distal comb has similarly the standard error 0.2890 and the *X* score mean, having a variance of 0.2193, has a standard error of 0.4683.

Now misclassification will occur when a strain mean deviates from its racial mean by at least half the difference between the means of the two races, provided that the deviation is in the right direction, *viz.*, that of the other strain. The probability of finding a deviation as large or larger than this minimum difference can be found by dividing half the difference between the racial means by the standard error of the strain mean. This quotient is entered in a Table of Normal Deviates, such as is given by Fisher (1936a) or Mather (1938) and the probability of obtaining an equally large chance deviation is then found. We should note, however, that as deviations in but one of the two possible directions will lead to misclassification we must divide the probability given by the Table of Nor-



mal Deviates by two in order to obtain the probability we require.

The three quantities, proximal comb mean, distal comb mean and X score mean are analyzed in this way in Table 7.

TABLE 7  
MISCLASSIFICATION OF STRAINS

	Means		Difference	Standard error	Ratio
	Race A	Race B			
Proximal comb ....	6.4655	5.5500	0.9155	0.3569	1.283
Distal " ....	5.3236	4.7267	0.5969	0.2890	1.033
X score .....	9.1001	7.8892	1.2109	0.4683	1.293

The probability of obtaining by chance deviations as large or larger than the one under consideration decreases as the ratio of the particular deviation to the standard error increases. Then it is clear that both the X score and the proximal comb are better for classification of races A and B than is the distal comb. Further, the X score enjoys a small but definite advantage over the proximal comb. It would indeed be surprising if this were not the case, as  $\lambda_p$  and  $\lambda_d$  were chosen to maximize the racial difference.

The Table of Normal Deviates gives the probability corresponding to a ratio of 1.293 as just less than 0.20. Dividing this by 2 we obtain as the frequency of misclassification a value just under 0.10. For various reasons discussed by Fisher, this is a maximum value for misclassification, but in our example there is no reason to expect that the real frequency will be much less than this calculated maximum.

Testing the frequency of misclassification empirically, by the calculation of the X score for each of the 23 strains of Table 4, we find that of the B strains none fall above the mid value of 8.495 and of the A strains only one falls below this value. Thus the actual misclassification in the strains used would be 0.043, a value somewhat below the expected frequency of 0.10. As only 23 strains were used, there is no reason to suspect a real difference between observed and expected misclassification.

(b) *Wing and Leg Measurements.* In each of the 20 strains, 10 of each race, marked with an asterisk in Table 1, the length and width of the right wing ( $x_l$  and  $x_w$ ) and the length of the right tibia ( $x_t$ ) was measured on each of 25 males and 25 females. The number of teeth in the proximal sex combs of the males was also recorded, as noted above. Table 8 gives the means of these measurements for each strain. Each unit is approximately 39  $\mu$ . In Table 9 will be found the sums of squares and cross products of deviations of the strain means of the various measurements from the racial means. These correspond to the sums of squares and cross products used in obtaining  $\lambda_p$  and  $\lambda_d$  in the previous section.

TABLE 8  
WING AND LEG MEASUREMENTS

	Females			Males			
	$\bar{x}_l$	$\bar{x}_w$	$\bar{x}_t$	$\bar{x}_l$	$\bar{x}_w$	$\bar{x}_t$	$\bar{x}_p$
<b>Race A :</b>							
Olympic-2 .....	48.14	28.70	18.94	42.02	24.86	17.46	6.64
Crater Lake-3 .....	44.72	25.92	17.56	41.64	24.32	17.20	6.76
Zuni-8 .....	45.86	26.60	17.20	44.06	23.98	16.22	6.48
Santa Rita-1 .....	43.16	24.62	16.28	39.16	22.26	15.60	6.04
Julian I-6 .....	44.88	26.30	17.38	41.10	23.90	16.90	6.88
Cuernavaca-8 .....	44.72	24.96	16.20	41.56	23.06	15.86	5.96
Pike's Peak-4 .....	43.90	25.32	16.56	41.88	24.40	16.74	6.08
Oaxaca-4 .....	46.76	26.64	18.48	41.68	24.02	17.12	6.40
Black Hills-5 .....	40.86	23.66	16.10	37.12	21.32	15.34	6.80
Mara-3 .....	45.84	26.02	16.68	39.90	22.04	15.30	6.36
Racial mean .....	44.884	25.874	17.138	41.012	23.416	16.376	6.440
<b>Race B :</b>							
Reedsport-2 .....	43.38	26.24	17.10	39.52	23.90	15.94	5.64
Campbell River-3 ..	44.90	25.88	16.66	39.08	22.02	14.04	5.88
Quilcene-4 .....	45.28	26.68	17.10	38.84	22.60	15.24	5.76
Quesnel-5 .....	46.54	27.24	17.46	42.00	24.64	16.14	5.72
Sisters-9 .....	45.34	27.04	17.48	41.64	25.06	16.32	6.08
Lassen-2 .....	44.08	25.32	16.80	39.50	22.80	15.80	5.36
Sequoia-14 .....	45.04	25.72	16.92	40.36	22.52	15.92	5.88
Nojogui-8 .....	43.68	24.36	16.68	39.44	22.22	15.86	5.76
Wawona-4 .....	46.24	26.52	17.44	41.58	23.36	15.84	5.92
Merritt-4 .....	46.56	26.36	17.02	41.82	23.38	15.86	5.20
Racial mean .....	45.104	26.136	17.066	40.378	23.250	15.786	5.720
All means based on 25 individuals.							

It will be noticed that whereas the wing length and width are highly correlated both with one another and with tibia length, none of these three measurements is correlated with the number of teeth in the proximal sex comb. Hence we may inquire whether the wing and leg measurements contribute any information as to racial difference and if

TABLE 9  
SUMS OF SQUARES AND CROSS PRODUCTS OF WING AND LEG MEASUREMENTS

	Wing length	Wing width	Tibia length	Sex comb teeth
<b>Females:</b>				
Wing length .....	47.8276	29.1644	16.1991	.....
Wing width .....	29.1644	23.5206	12.8045	.....
Tibia length .....	16.1991	12.8045	9.4028	.....
<b>Males:</b>				
Wing length .....	46.2078	24.9475	11.4402	- 0.5904
Wing width .....	24.9475	21.9288	11.0084	0.7592
Tibia length .....	11.4402	11.0084	7.3766	0.8368
Sex comb teeth ....	- 0.5904	0.7592	0.8368	1.6256

so whether they may be profitably combined with the sex comb values for this purpose.

Adopting the same statistical technique as before we find the coefficients  $\lambda_l$ ,  $\lambda_w$  and  $\lambda_t$  which will maximize the difference between the females of the two races when used in the function

$$\bar{X} = \lambda_l \bar{x}_l + \lambda_w \bar{x}_w + \lambda_t \bar{x}_t$$

The racial differences in these measurements, from Table 8, and the sums of squares and cross products from Table 9, give the following as the equations of estimation of these coefficients:

$$47.8276\lambda_l + 29.1644\lambda_w + 16.1991\lambda_t = -0.220$$

$$29.1644\lambda_l + 23.5206\lambda_w + 12.8045\lambda_t = -0.262$$

$$16.1991\lambda_l + 12.8045\lambda_w + 9.4028\lambda_t = 0.072$$

Then

$$\lambda_l = 0.00658, \quad \lambda_w = -0.06686, \quad \lambda_t = 0.08738$$

and

$$D = 0.02236$$

where  $D$  is the mean difference in score between the races.

The analysis of variance between and within races may be simply performed by putting the sum of squares within races equal to 1, and the sum of squares between races

equal to  $\frac{n_A n_B}{n_A + n_B} D$ , i.e.,  $5 \times 0.022362$ .

We note that there are 19 degrees of freedom in all and that 3 will be taken up by the inter-racial difference because we have fitted two adjustable parameters to the data. The analysis is then:

	Sum of squares	Degrees of freedom	Mean square
Races .....	0.1118	3	0.0373
Strains .....	1.000	16	0.0625

and the racial difference is clearly not significant.

A similar calculation for the wing and leg measurements of the males gives:

$$\begin{aligned} 46.2078\lambda_l + 24.9475\lambda_w + 11.4402\lambda_t &= 0.634 \\ 24.9475\lambda_l + 21.9288\lambda_w + 11.0084\lambda_t &= 0.166 \\ 11.4402\lambda_l + 11.0084\lambda_w + 7.3766\lambda_t &= 0.590 \end{aligned}$$

Then

$$\lambda_l = 0.0432, \quad \lambda_w = -0.1917, \quad \lambda_t = 0.2991$$

and

$$D = 0.1720$$

The analysis of variance is then:

	Sums of squares	Degree of freedom	Mean square
Races .....	0.8601	3	0.2867
Strains .....	1.0000	16	0.0625

and  $z = 0.7617$ .

This is almost equal to the value of  $z$  at the 1 per cent. point and so indicates a significant racial difference.

Thus even though these measurements detect no racial difference in the females, they serve to detect a difference when males are used.

We may then profitably combine these measurements with the counts of teeth in the proximal sex combs. The equations for the estimation of the  $D$  are from Tables 8 and 9.

$$\begin{aligned} 46.2078\lambda_l + 24.9475\lambda_w + 11.4402\lambda_t - 0.5904\lambda_p &= 0.634 \\ 24.9475\lambda_l + 21.9288\lambda_w + 11.0084\lambda_t + 0.7592\lambda_p &= 0.166 \\ 11.4402\lambda_l + 11.0084\lambda_w + 7.3766\lambda_t + 0.8368\lambda_p &= 0.590 \\ -0.5904\lambda_l + 0.7592\lambda_w + 0.8368\lambda_t + 1.6256\lambda_p &= 0.720 \end{aligned}$$

giving

$$\lambda_l = 0.0746, \quad \lambda_w = -0.1962, \quad \lambda_t = 0.2053, \quad \lambda_p = 0.4560$$

and

$$D = 0.4642$$

Now as we have fitted an extra parameter in obtaining this discriminant we have 4 degrees of freedom between the races, leaving but 15 between strains within the races. The analysis of variance is thus:

	Sums of squares	Degrees of freedom	Mean square
Races .....	2.3209	4	0.5802
Strains .....	1.0000	15	0.0667

$z$  is 1.0815, which indicates a significant difference as its value at the 1 per cent. point is 0.7939.

We may test the increase in efficacy of classification when using this score over that obtained when the proximal comb values are used alone, by calculating the frequency of misclassification in the same way as before. The sex comb misclassification must now be calculated from the 20 strains used in these experiments and not from the 23 used in the previous section in order that it shall be comparable with the results from using the combined comb, leg and wing measurements. Then by the method of the previous section we find:

	Difference	Standard error	Ratio
Sex comb .....	0.720	0.293	1.229
Score .....	0.464	0.176	1.318

The use of the extra measurements has increased the precision of our classification and the increase is greater than that obtained by the use of the distal combs in addition to the proximal comb (see above). Thus of the measurements taken in these experiments the most accurate classification, or, alternatively, the most significant difference between the races, is obtained when using the proximal comb, the wing length and width and the tibia length. As, however, these last three measurements are more difficult to obtain, and, in any case, less accurate, it may be felt that in the majority of cases the slight increase in accuracy obtainable by their aid does not justify the extra difficulty of taking them. The numbers of teeth in the proximal and distal combs are very easy to count and are probably in general preferable.

TABLE 10

	First experiment		Second experiment	
	$\bar{x}_p$	$S(x_p^2)$	$\bar{x}_p$	$S(x_p^2)$
Santa Rita-1 (A) .....	6.40	6.60	6.04	4.96
Pike's Peak-4 (A) .....	5.92	5.84	6.08	7.84
Reedsport-2 (B) .....	5.48	6.24	5.64	5.76
Campbell R.-3 (B) .....	5.76	6.56	5.88	2.64

(c) *The Consistency of the Racial Difference.* We may finally inquire into the consistency of the difference in

external morphology between the races, as revealed by these measurements. We have two sets of data suitable for testing this point with reference to the sex combs.

In the first place it will be remembered that four strains, two of each race, were used in both of the experiments that were carried out. In each of these experiments the numbers of teeth in the proximal sex combs of 25 males were obtained (*cf.* Table 1). Now each of these counts was made on flies raised under the conditions described in Section 2, and in particular all were raised at 24.5° C. Hence both experiments should give substantially the same result for the racial difference in proximal sex comb teeth if the technique is suitable and the difference real.

The mean numbers of proximal sex comb teeth, based on 25 males, is given for each of these four strains in Table 10, as is the sum of squares of deviations of the individuals round the strain mean. There are two sets of values for each strain, one from each of different experiments. We may extract the desired information from the data by an analysis of variance. As there are four strains each comprising 25 individuals from each experiment, we have a total of 200 flies in all, and thus 199 degrees of freedom in the whole analysis. Of these 7 degrees of freedom will be concerned with the differences between the 8 means, and the remaining 192 will be concerned with the differences between individuals of the same strain on the same occasion. The 7 degrees of freedom between the means may be further subdivided into 1 for the difference between the two experiments, 1 for the difference between the races averaged over both experiments, 1 for the variation of the racial difference with the experiments, *i.e.*, the "interaction" of races and experiments, and 4 for the differences between strains of the same race and the interaction of these differences with experiments. These last four may be lumped, as we are not particularly interested in them. We use the arithmetical technique described particularly by Fisher (1936b) in analyzing the data and obtain the following analysis (Table 11):

TABLE 11

	Sums of squares	Degrees of freedom	Mean square
Experiments .....	0.02	1	0.02
Races .....	8.82	1	8.82
Race-Exp. Interaction .....	0.72	1	0.72
Strains and Strain-Exp. Interaction .....	4.60	4	1.15
Individuals .....	45.84	192	0.2388
Total .....	60.00	199	

It is clear that there is no real difference between the experiments nor is there any indication of an interaction between the racial difference and the experiments. In this way our belief in the reality of the racial difference in the proximal sex comb must be considerably strengthened.

The second test of the validity of the racial discrimination is in some way more stringent and of more interest. Samples of eight of the strains, four from each race, were raised at two different temperatures. The parents of the experimental flies were allowed to lay eggs at 24.5° C., as usual, and as before, were transferred to fresh bottles each day. When the eggs were laid, however, one set of bottles was allowed to develop at that same temperature of 24.5° C., and the results from these have, in fact, been incorporated in the analysis of Section 3 (a). Another set from the same parents was removed to a cold room with a temperature of approximately 17.5° C. These latter were subjected to temperature fluctuations of rather greater magnitude than is usual in an incubator, but even this variation is considerably smaller than the difference between the two temperatures used on the two sets of flies. Apart from the temperature difference the conditions, *e.g.*,

TABLE 12

	Cold			Warm		
	$\bar{x}_p$	$\bar{x}_d$	$\bar{x}$	$\bar{x}_p$	$\bar{x}_d$	$\bar{x}$
A. Wawona-6 .....	6.76	5.24	9.3480	6.72	5.08	9.2341
Pike's Peak-4 .....	6.60	5.40	9.2727	5.92	5.12	8.4539
Tree Line-3 .....	7.36	5.92	10.2898	6.56	5.56	9.3116
Santa Rita-1 .....	7.08	5.72	9.9108	6.40	5.16	8.9537
B. Sequoia-5 .....	5.96	5.56	8.7116	5.72	5.04	8.2143
Reedsport-2 .....	5.84	5.00	8.3145	5.48	4.20	7.5589
Campbell R.-3 .....	6.08	5.00	8.5545	5.76	4.80	8.1355
Cowichan-6 .....	5.36	5.08	7.8741	4.56	4.04	6.5594

number of flies per bottle, etc., were substantially the same.

The means of the proximal and distal comb teeth for all eight strains are given in Table 12. The cold series on the whole show more teeth per comb both proximally and distally. The variance of the discriminant suitable for combining the results of the two combs, as found in Section 3 (a), is analyzed in Table 13. There are 400 flies and hence 399 degrees of freedom in all. Of these, 384 are solely concerned with variations of individuals round the means of their strains. The difference between the warm and cold results takes up 1 degree of freedom, as do the racial difference and the interaction of the racial difference with the temperatures. The allocation of but 1 degree of freedom to the racial difference is not quite exact. It would be correct if the discriminant function used had been obtained from completely independent data. If the discriminant had been calculated from all the data, but no other, of these two experiments the racial difference would take 2 degrees of freedom. In actual practice half the data of this analysis, viz., the warm series, has formed  $\frac{8}{23}$  of the results from which the discriminant was calculated. Hence neither 1 nor 2 degrees of freedom can strictly be allocated to the result. We shall not be far wrong, however, in taking the correct value as one, and in any case, as will be seen below, the results of the analysis are not seriously altered whether we take 1 or 2. There are 6 degrees of freedom for the variation of the strains round the racial means and also 6 for the interaction of this strain variation with temperature. This completely accounts for the 399 degrees of freedom of the analysis. We obtain the results shown in Table 13.

Again there is a large difference between the races whether 1 or 2 degrees of freedom are used, but it is now accompanied by a difference between the two experiments, as might be expected inasmuch as they were conducted at different temperatures. What is perhaps more impor-



TABLE 13  
ANALYSIS OF VARIANCE OF THE X SCORE IN TEMPERATURE EXPERIMENTS

	Sums of squares	Degrees of freedom	Mean square
Experiments .....	53.6551	1	53.6551
Races .....	184.1832	1	184.1832
Race-Exp. Interaction .....	0.0202	1	0.0202
Strains .....	70.0283	6	11.6714
Strain-Exp. Interaction .....	12.3230	6	2.0538
Individuals .....	129.9914	384	0.3385
Total .....	<b>450.2012</b>	<b>399</b>	

tant, however, is that there is no evidence for interaction of the racial difference with temperature. In other words, the racial difference is not only real at both temperatures but it is the same at both temperatures. Neither is there any very large interaction between strains and temperature. Thus, in brief, we have found evidence of differences between the races of *Drosophila pseudoobscura* in the two sex combs, proximal and distal, and also in the wing and leg lengths of the male flies, though none was apparent between the females. Further, the racial difference in sex combs was successfully repeated on distinct occasions using the same cultural technique, and has also been shown to occur, and to be substantially the same, at two very different temperatures, one of which would be more favorable for the development of race A and the other for race B. Thus we can say quite definitely that there do exist differences in external morphology between the races, though it is true that the innate variation between individuals and strains obscures the racial difference unless a suitable statistical technique is employed in the analysis.

#### 4. SUMMARY

The use of suitable statistical analyses permits the demonstration of racial differences in the numbers of the sex-comb teeth and in certain measurements of the legs and wings, when taken on male flies. No such differences were found between females of the two races.

The differences in numbers of sex-comb teeth were constant over a series of experiments even when performed at different temperatures.

The use of these criteria for assignation of strains to the two races would result in 10 per cent. misclassification.

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# BUILDING STONES TO A CHEMISTRY OF EVOLUTION

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THE rule of inheritance is that like begets like. This holds for the largest as well as the smallest organisms. When an alga divides, there are two algae. There is twice as much water, twice as much minerals, twice as much protoplasm, enzymes, membranes, carbohydrates. For each molecule in the parent cell, there are two now. The same is ultimately true in the reproduction of higher plants and animals; we have a doubling (or trebling or multiple formation) of the parent organism, practically molecule for molecule. This is a most unusual event from the view-point of the chemist, because in a chemical reaction the original compounds never double themselves; quite to the contrary, they disappear and something entirely different appears in their place.

Evolution is a deviation from inheritance. As ordinarily interpreted, it means (at least ultimately) the development of more complex organisms from simpler ones. This can be brought about only by a change in the hereditary units. Evolution, then, means the acquisition of more hereditary units, and of new, different ones. The only hereditary units considered in this paper are the genes. The chemical laws which apply to them will very probably apply to other hereditary mechanisms. Evolution by loss of hereditary units is likely to be a rare case. Loss of genes does not necessarily require chemical reactions, and this possibility shall not be considered here.

Through irregular cell division, through fusion of wounded cells or by other means (*e.g.*, colchicine acid), a cell may acquire more than its normal share of genes. A complete second set of genes or part of a second set may be added to a cell. This increase in the number of genes is caused primarily by a biological event whose frequency

of occurrence is determined by the frequency of irregular cell divisions, of wounding, etc. No chemical law can be applied to this phase of evolution.

The changes brought about by addition of parts of chromosomes or one or more entire sets of chromosomes are not very great, and the range of variation is limited. Great, decisive changes can be produced only by the creation of new genes. The making of a new gene is primarily a chemical event; the chemical reaction precedes the biological consequences. It shall be assumed here, for simplicity's sake, that a gene consists of one giant molecule (see P. Jordan, 1938). All chemical laws would apply also if the genes are multimolecular; the reaction would appear much more complex to us, but in nature, a multimolecular reaction proceeds just as easily as a unimolecular one.

Since the chemical composition of genes is entirely unknown, we can not say by what chemical mechanism the dividing cell manages to double all the genes. We only know that a gene once destroyed in a cell can never be replaced, and its offspring has to carry on forever without this gene (if the cell can live at all without it). This means that each gene can only be produced by itself, possibly with the assistance of other genes or protoplasm molecules. Without this gene, all other molecules in the cell can not reproduce it. The probability that a lost gene is reconstructed by the other molecules of the nucleus is zero, according to experimental genetics.

A gene must be an extremely complicated molecule. The creation of such a structure from simple food molecules is as improbable as spontaneous generation. Since untold millions of different genes have originated in all the plant and animal species since the beginning of life on earth, the formation of a new kind of gene must be possible. There is reason to believe that genes have a certain basic chemical structure in common. To the chemist, the appearance of a new gene indicates most likely a change of an already existing gene, the addition of some molecular group or

the loss of one. We might think of side chains in Ehrlich's sense. This chemical conception of the process which yields new genes agrees fortunately with the experiences of the biologist. Haldane (1932, p. 108) states:

My own quite speculative theory of orthogenetic evolution . . . is that we are dealing here not only with the accumulation of genes having similar action, but with the very slow modification of single genes, each changing in turn into a series of multiple allelomorphs. The phrase "modification of the gene" is of course a rather misleading simplification. What I mean is that mutation was constantly modifying the gene, and that at any given time, natural selection acted so as to favor one particular grade of modification at the expense of others.

To such a chemical reaction of the gene, the law of mass action can be applied which states that the rate of reaction (here the frequency of a certain change) is proportional to the concentration of each reacting component. Only one gene in a cell will react at any time. The probability that two react simultaneously is very, very small. The other reacting component is unknown, though it must be a cell constituent. Since nothing is known about the other component, we must be satisfied with the statement that the rate of change of a gene is proportional to the concentration of that gene. Since there is only one gene of each kind per gamete, the rate can not be proportional to the  $n$ th power; it must be directly proportional to the gene concentration, except in polyploids.

Among the many factors influencing the rate of chemical reactions, temperature is quite important. An increase in temperature will accelerate the chemical process.

The rate may further be influenced by changes in the stratum where the reaction takes place. The change from light to dark is very important since light is capable of catalyzing many chemical reactions. The amount of available oxygen varies greatly at different depths of lakes and oceans. Rates of reaction are frequently affected by changes in concentration of electrolytes, especially of H ions, but of other ions as well, and even of non-electrolytes such as sugar, or protein.

We must expect, therefore, an increase in the creation of new genes from old: (1) by an increase in the concentra-

tion of genes on earth; (2) by an increase of temperature; (3) by a change of environment.

### (1) THE RÔLE OF GENE CONCENTRATION

The average cell has 2 equal sets of chromosomes, each with  $n$  genes. If an organism has  $x$  cells, it will have  $2 nx$  genes and if there are  $y$  individuals of this species on earth, the "concentration" of all genes of this organism on earth is  $2nxy$ . All these chromosomes have a chance for variation, *i.e.*, for abnormal chemical reaction, but if such a change occurred in the somatic cells, it would be of no influence upon evolution in the case of animals, and very rarely only in plants. If it happened in gametes, it would be significant only in the case where this gamete developed into a new organism. Thus, the chance that a chemical change affects evolution is really proportional to the offspring produced over a certain period, *e.g.*, one year, or really to twice the offspring, since each gamete has equal chances.

According to Statistical Abstracts of the United States, 1934, there is in this country the following female population:

White	.....	53,700,000	=	3,550	times that of the Chinese
Negro	.....	6,035,000	=	399	" " " " "
Indian	.....	162,047	=	10.7	" " " " "
Japanese	.....	57,063	=	3.8	" " " " "
Chinese	.....	15,152	=	1	" " " " "

Though there are slight differences in regard to number of children per mother, the above numbers represent fairly accurately the relative number of gametes which might undergo a chemical change. It does not represent the relative chances for mutation, for the rate of reaction in different races may be different. Certain races of *Drosophila* mutate much more easily than others.

Nevertheless, such numbers are instructive. The hens in U.S.A. laid 32,000,000,000 eggs in 1934, but only 673,000,000 chickens were raised. Consequently, the latter number is the factor for the chance of mutation.

According to Haldane (1932, p. 104), the number of wheat plants on earth is about  $5 \times 10^{14}$ , and this is the

factor for the evolutionary chance, not the  $2 \times 10^{16}$  seeds that were obtained from them. If we compare this number of plants with that of a rare orchid of which only perhaps 100,000 plants might exist on earth, the ratio is 5,000,000,000:1.

This is the significance of the concentration of genes on earth. If we assume that a gene in the rare orchid mutates once in 10 million plants, and in wheat much more slowly, perhaps once in 1,000 million plants, we would still have 500,000 mutations per year in wheat, and only one every century in the orchid. The concentration of genes, *i.e.*, the number of individuals of a species, is an important factor in evolution. When the differences in frequency of individuals are slight, this loses its meaning because of the many other factors affecting mutation, but when the differences are enormous, it must become significant. It should be possible to test this claim statistically. This can not be done with only a few species or families because different genes in different plants will have different rates of reaction. But if we consider all plants in the United States, this should be a number large enough to test the theory. Heller's "Catalogue of North American Plants North of Mexico" (1900) lists 16,673 species in 209 families. The families are arranged on the following pages according to the number of species.

*Families with only 1 species:* Ceratopteris, Mayaca, Croomia, Gyrotheca, Dioscorea, Casuarina, Leitneria, Apodanthes, Batis, Anredera, Tropaeolum, Hippocratea, Clusia, Canella, Koeberlinia, Carica, Datisca, Punica, Trapa, Symplocos, Sesamum, Phryma, Adoxa, Scaveola;

*Families with 2 species:* Trichomanes, Zamia, Canna, Marantha, Saururus, Anemiopsis, Peperomia, Schoepfia, Ximenia, Ceratophyllum, Podostemon, Crossosoma, Bursera, Melia, Swietenia, Pachysandra, Simondsia, Staphylea, Impatiens, Tamarix, Fouquieria, Amoreuxia, Piriqueta, Turnera, Pholisma, Ammobroma, Diospyros;

*Families with 3 species:* Osmunda, Pypha, Apteris, Burmannia, Butneria, Platanus, Empetrum, Corema, Ceratiola, Cyrilla, Cliftonia, Daphne, Dirca, Clethra;

*Families with 4 species:* Schizaea, Ornithopteris, Lygodium, Azolla, Salvinia, Halophila, Philotria, Phytolacca, Rivina, Petiveria, Menispermum, Cebatha, Hamamelis, Fothergilla, Liquidambar, Gordonia, Stuartia, Frankenia, Diapensia, Pyxidanthera, Shortia, Galax, Myrsine, Jacquinia, Icacora, Martynia;

*Families with 5 species:* Taxus, Tumion, Reseda, Oligomeris, Dipetalia, Elaeagnus, Lepargyrea, Menyanthes, Nephrophyllidium, Limnanthemum, Dipsacus, Knautia, Scabiosa;

*Families with 6 species:* Marsilia, Piluria, Triglochin, Scheuchzeria, Lilaea, Pontederia, Piaropus, Heteranthera, Buckleya, Nestronia, Comandra, Pyrolaria, Simarouba, Suriana, Castela, Holacantha, Ailanthus, Picramnia;

*Families with 7 species:* Ephedra, Malpighia, Janusia, Aspicarpa, Thryallis, Byrsonimia, Tilia, Corchorus, Triumfetta, Elatine, Bergia, Terminalia, Conocarpus, Laguncularia, Bignonia, Chilopsis, Catalpa, Campsis, Stenolobium; Crescentia;

*Families with 8 species:* Najas, Eriocaulon, Dupatya, Lachnocaulon, Myrica, Comptonia, Sarracenia, Chrysamphora, Drosera, Dionaea, Parnassia, Aesculus, Plumbago, Statice, Limonium, Styrax, Mohrodendron.

Of the following groups, only the family names shall be mentioned:

Number of species  
per family

9	Limnanthaceae
10	Sparganiaceae, Lauraceae
11	Moraceae, Melastomaceae
12	Callitrichaceae, Passifloraceae
13	Aizoaceae, Stemliaceae, Araliaceae, Monotropaceae
14	Lemnaceae
15	Zygophyllaceae
16	Selaginellaceae, Bromeliaceae, Sapindaceae
17	Urticaceae, Loranthaceae, Rhizophoraceae, Haloragidaceae, Sapotaceae
18	Juglandaceae, Loganiaceae
19	Xyridaceae, Ulmaceae, Nymphaeaceae, Aceraceae, Pyrolaceae, Orobanchaceae
20	Lycopodiaceae, Magnoliaceae
23	Equisetaceae, Berberidaceae, Aquifoliaceae
24	Ophioglossaceae, Oxalidaceae
25	Fumariaceae
26	Rutaceae, Anacardiaceae, Cistaceae
27	Isoetaceae
28	Betulaceae, Aristolochiaceae, Hydrangeaceae, Celastraceae, Lythraceae, Lentibulariaceae
31 to 100	38 families

The theory claims that very common plants should show much variation, *i.e.*, many species, while very rare plants should show little variation, *i.e.*, few species. No strict parallelism can be expected. Many deviations will occur because each species has its own rate of mutation. But 16,000 species is such a large number that individual differences should not affect the grand averages. The difficulty of the proof lies in the impossibility of getting anything



The following are the families with more than 100 species:

Species	Family	Species	Family
105	Violaceae	258	Polemoniaceae
106	Pinaceae	266	Onagraceae
111	Gentianaceae	281	Clchoriaceae
116	Juncaceae	291	Boraginaceae
130	Asclepiadaceae	297	Caryophyllaceae
130	Chenopodiaceae	331	Umbelliferae
130	Orchidaceae	336	Labiatae
131	Rubiaceae	370	Polygonaceae
143	Solanaceae	373	Liliaceae
146	Salicaceae	373	Ranunculaceae
164	Ericaceae	529	Cruciferae
170	Polypodiaceae	558	Rosaceae
170	Saxifragaceae	627	Scrophulariaceae
189	Hydrophyllaceae	770	Cyperaceae
194	Cactaceae	1226	Papilionaceae
211	Malvaceae	1230	Gramineae
250	Euphorbiaceae	2631	Compositae

but a very rough and rather personal conception of the relative frequency of various species on a continent. Plants may be frequent in certain climatic regions or on certain soils, and entirely missing in the rest of the United States. It must further be remembered that with trees and perennials generally, only the number of new plants originating from seed can be counted. That eliminates most trees as "common plants" (Aceraceae, 19 species; Betulaceae, 28 species; Pinaceae, 106 species).

It will probably be admitted by most readers that practically all families with less than 10 species are rare, that the families with more than 1,000 species are the commonest plants, to be found in every locality on every soil.

Far more common than the commonest of all these plants are certain bacteria. *Bacterium coli* lives in the intestine of man and all mammals. It is excreted by man at the rate of about  $25 \times 10^{10}$  cells per capita per day, = nearly  $10^{14}$  cells per year. Multiplied with the number of people on earth, this means more than  $10^{23}$  individuals growing in man per year. To that must be added the growth in all mammals. Other intestinal bacteria (Streptococci, Lactobacilli, Bacteroides) will occur in numbers higher than  $10^{20}$ . On the surface of all plants are regularly found *Bacterium herbicola* and *Pseudomonas fluorescens*. From the data given by Duggeli, the number of these bacteria on the surface of all plants in United States

must far exceed  $10^{21}$  individuals. These numbers are 10 million to 1,000 million times that of all the wheat plants on earth. Among bacteria, we should expect the greatest frequency of mutations. That this is actually the experience of bacteriologists, to such an extent as to make taxonomy almost impossible, shall be shown in the last chapter.

The above-mentioned bacteria are not exceptions. There are millions of bacteria and thousands of protozoa in every gram of fertile soil; there are thousands of bacteria to every cc of surface water. Their rate of multiplication in nature is unknown. In this respect, the intestinal bacteria are probably exceptionally favored.

## (2) THE RÔLE OF TEMPERATURE

It is known to all physiologists that the rate of chemical reactions is increased by a rise of temperature, and that this law applies to reactions *in vivo* as well as *in vitro*. However, there is a striking exception in photochemical reactions which proceed at the same rate at all temperatures. This is quite important in our case, for it affords us the opportunity to test certain theories of the cause of evolution. In the preceding pages, the creation of new genes has been described as a chemical reaction. It has been frequently stated in recent years that radiation—be it ultra-violet, radioactive or cosmic in origin—may be the immediate cause of changes in the hereditary units of a species, and thereby the ultimate cause of evolution.

It seems possible to test this claim statistically. If evolution is caused by chemical changes of the common type which take place in the dark as well as in light, then evolution should go on more rapidly at the tropics than in moderate climates, and we should find the largest number of species of any one group near the equator. Exceptions would be mammals and birds whose temperature is independent of the climate. If, however, cosmic rays were the cause of evolution, temperature should have no great influence on species formation.

Of great interest in this respect is an investigation by Tischler (1934) into the frequency of polyploids in differ-

ent climates. Although the chromosomal composition of Sicilian plants is known for only one third of all species, and for Iceland and Faroe Islands for only about one half of the species, the following table is quite instructive.

	Square kilo- meters	Lati- tude	Number of species			Percentage of polyploids		
			Dicots	Monocots	Total Angiosperms	Dicots	Monocots	Total Angiosperms
Iceland .....	103,000	66-70°	239	120	359	47.4	71.9	54.5
Faroe Islands .....	1,400	63°	172	95	267	42.7	63.5	49.4
Schleswig-Holstein ..	23,000	54-55°	777	293	1,070	39.0	60.0	44.1
Sicily .....	25,000	37-38°	1,800	545	2,345	26.0	48.8	31.3

The left half of the table shows a decided increase in the number of species with a decrease in latitude, *i.e.*, with an increase in temperature. This suggests that the creation of new species is favored by an increase in temperature. The right half of the table shows that polyploidy (which has been explained above as not being caused by chemical reactions) is not favored by an increase in temperature, but actually increases distinctly in the colder climates. This was also emphasized by the fact that those plants of Schleswig-Holstein which are found also in northern countries consist to 60 per cent. of polyploids (127 species) while those which are found in southern countries contain only 27 per cent. polyploids (70 species). Whether the cause of polyploidy be cosmic radiation or an adaptation mechanism for growth at lower temperatures, the cause is evidently different from the main species-forming force of evolution, which is chemical in nature and increases in activity with higher temperatures.

It might be doubted that the regions selected are large enough to use them for such statistical surveys. Since the angiosperms represent by far the largest part of the flora of any country, they may well be considered representative. A second example shall be chosen from zoology, namely the reptiles.

This group has been chosen because it is compiled quite comprehensively in Ditmars's book, "Reptiles of the World" (London, 1910). Though quite a large number of species have not been given in sufficient detail, there was no doubt in the large majority of cases whether a species lived in a temperate or a hot climate. In doubtful cases, the decision was made in favor of temperate climate. South Africa and southern South America were considered temperate, also California and New Mexico, while Mexico and southern Europe were considered hot. The result of the compilation was as follows:

	Number of species		
	In moderate climates	In hot climates	Undecided
Chelonia (turtles) .....	50	135	30
Crocodylia (alligators) .....	0	23	0
Lacertilia (lizards) .....	120	1,408	89
Ophidia (snakes) .....	165	1,219	226
Total reptiles .....	<b>335</b>	<b>2,785</b>	<b>345</b>
Percentage distribution ..	10 per cent.	80 per cent.	10 per cent.

Even if all the undecided species were counted as belonging to the moderate climates, they would still represent less than one fourth of those in hot climates. The agreement with Tischler's statistical results with plants is rather convincing.

I have been told by zoologists that a similar, probably even greater majority of species in hot climates exists in the fishes and among insects, but I have found no material that could be treated without a very great amount of work. The difference should not be so great among the warm-blooded animals, the mammals and birds, because the genes are kept at uniform body temperature.

An analysis has been attempted by classifying the mammals listed in Trouessart's "Catalogus Mammalium," 1898-9. The result is at first glance not much different from that of the reptiles for it shows a distinct majority of tropical species. But with reptiles, the species of moderate climates averaged 10 per cent., and with mammals 26 per cent. of the total. The outspoken dominance of

## DISTRIBUTION OF ALL MAMMALS BETWEEN TROPICAL AND TEMPERATE CLIMATES

	Number of species				Total living species	Percentage of living species		
	Extinct	Tropical	Moderate	Undecided		Tropical	Moderate	Undecided
Bimana .....	28	207	..	1	1	..	100	
Primates .....	85	49	1	1	228	91	2	7
Prosimia .....	36	396	42	83	51	96	2	2
Chiroptera .....	72	160	84	30	521	76	8	16
Insectivora .....	516	143	111	68	274	58	31	11
Carnivora .....	23	2	21	7	322	44	35	21
Pinnipedia .....	435	759	525	184	30	7	70	23
Rodentia .....	38	0	0	0	1,468	52	36	12
Tillidontia .....	1,428	201	79	70	0	0	0	0
Ungulata .....	31	6	1	0	350	57	23	20
Sirenia .....	290	26	51	35	7	86	14	0
Cetacea .....	334	42	2	6	112	23	45	32
Edentata .....	248	84	27	73	50	84	4	12
Marsupialia .....	49	0	0	0	184	45	15	40
Allotheria .....	10	1	0	2	0	0	0	0
Monotremata .....					3	33	0	67
Total mammals .....	3,623	2,076	948	577	3,601	58	26	16

tropical species in mammals is limited to the monkeys and the bats, about one fourth of all species, while the snakes, lizards and crocodiles represent 94 per cent. of all reptiles. The table comparing only those species with outspoken climatic preferences, shows this difference very distinctly. The grand average ratio of species in tropical and moderate climates is with mammals 69:31, *i.e.*, 2:1 and with reptiles 9:1.

A preponderance of species in tropical climates must be expected even with mammals for the simple reason that with the abundant food, the number of individuals is considerably larger. The effect of the number of individuals on the frequency of variation has just been discussed. While the above evidence in regard to mammals can not be considered absolute proof, the ratio of species in hot and cool climates, which is so very different with cold-blooded and warm-blooded animals, suggests very strongly a strong influence of temperature upon the rate of evolution.

## (3) THE RÔLE OF ENVIRONMENT

It is generally assumed that the slow geological changes causing a gradual change of climate, together with the

## RATIO OF SPECIES IN TROPICAL AND TEMPERATE CLIMATES, AFTER OMISSION OF UNDECIDED CASES

Mammalia				Reptilia			
Species consid- ered		Tropical	Temperate	Species consid- ered		Tropical	Temperate
1	Monotremata . . .	100	0	23	Crocodylia . . . .	100	0
50	Prosimia . . . . .	98	2				
211	Primates . . . . .	98	2				
44	Edentata . . . . .	95	5				
438	Chiroptera . . . . .	91	9	1,528	Lacertilia . . . . .	92	8
7	Sirenia . . . . .	86	14	1,384	Ophidia . . . . .	88	12
111	Marsupialia . . . .	76	24	185	Chelonina . . . . .	73	27
280	Ungulata . . . . .	72	28				
244	Insectivora . . . .	66	34				
1,284	Rodentia . . . . .	59	41				
254	Carnivora . . . . .	56	44				
77	Cetacea . . . . .	34	66				
30	Pinnipedia . . . . .	9	91				
Average . . . . .		69	31	Average . . . . .		90	10

glacial periods, have been important factors in evolution. When a moist climate gradually becomes arid, the cell sap in plants may become more concentrated and thus cause a reaction which was impossible before. The decrease of fog and clouds may permit very short ultra-violet light to strike the seeds of plants or eggs of insects, and a reaction may thus take place hitherto unknown in that group of organisms. Such climatic changes may be sudden, as the drying of a swamp or salt marsh, but usually require many thousands or even millions of years.

Similar and even greater changes occur to some kinds of microscopic organisms far more frequently. Each gram of soil differs from the neighboring gram in its oxygen supply, in the amount of mineral and organic matter, in moisture content and exposure to sunshine. A large plant extends its roots over many cubic feet of soil and obtains the average effect, but to a microscopic plant like an alga, one gram is a universe. This soil climate of the alga will change with every rain, with every dry day, with a leaf falling on the ground. The algae, bacteria and protozoa of the soil will undergo continuous drastic changes of life conditions, and the chances for an abnormal reaction of any one of the genes is greatly increased.

An even greater change occurs daily at a stupendous scale to the bacteria commonly inhabiting the surface of plants, *Bacterium herbicola*, *Pseudomonas fluorescens* and in smaller quantities some of the *Lactobacilli* and *Streptococci* and other genera. They are swallowed by herbivorous animals at the rate of a hundred thousand individuals per gram of plant substance. This means a change from the dry, brightly lighted environment with abundant oxygen, but scant food on the outside of the leaves, to the dark, completely anaerobic intestine with an abundance of soluble food. There the bacteria remain for 1 to 7 days, depending upon the species of animal, and most of the bacteria die. The few survivors are thrown out, onto the soil or into water, together with an enormous number of intestinal bacteria which had been completely adapted to the uniform temperature and the rich food in the intestine, and which are bound to die in the new environment unless they can adapt themselves. This same change applies also to the infusoria swallowed by animals with the drinking water, and to the normal or pathological protozoic fauna of the animal intestine. This change of environment happens to untold billions of bacteria daily. It is only natural to suppose that entirely different food constituents passing through the cell membrane to the growth centers of the bacterial cell might occasionally, perhaps once in a trillion individuals, cause a variation in one gene, and thus produce a new variety.

Taxonomy of bacteria seems almost impossible because of the existence of nearly any imaginable intermediate form between any two established "species" (Rahn, 1920, 1928). We must either assume that these intermediates found "climates" or "universes" where they could multiply ever since they were first created or that they arise now and then by mutation from the "standard species." The latter assumption is far more probable, and can be supported experimentally.

Bacteria are so small and so simple in their forms that distinction of species within the genus is based largely on

biochemical properties, such as the ability to digest gelatin, or to ferment starch, sucrose, lactose, galactose, etc. These biochemical properties have been frequently found to vary. One of the first carefully studied cases is that of *Bact. coli mutabile* (Neisser and Massini). This species does not ferment lactose, and therefore produces white colonies on Endo agar. When these colonies are kept for a number of days, secondary colonies become visible in some of the old white colonies, and these new tiny colonies are red, *i.e.*, they can ferment lactose. The progeny from the red colonies "breeds true," it keeps the ability to ferment lactose even after many transfers on lactose-free media. The offspring of the white colonies continues to produce white colonies, with an occasional red secondary colony. This sounds like a simple case of occasional mutation of a single cell, and the frequency is about  $10^{-9}$ .

Quite similar is the observation of Twort with typhoid bacteria. While *Bact. typhosum* in the ordinary fermentation test will not attack dulcitol, it can be "trained" to ferment this sugar merely by cultivating it in dulcitol broth. Some strains will start to ferment this sugar after the third transfer, others not until the tenth transfer. The cultures then retain this ability for many transfers. The plate counts of the cultures suggest that a single cell, or a very few, acquire suddenly this ability and thus having more food available, outgrow the old strain.

While all strains of *Bact. typhosum* learned to ferment dulcitol, only one of all the strains tested by Twort and later by Penfold learned to ferment lactose. This is a very rare mutation, happening only perhaps once in  $10^{12}$  individuals.

It is not proved, however, that these mutations are really caused by the continuous chemical stimulation of the sugar concerned. It may be that the same mutation would have happened anyway, even in the absence of the special sugar; but since the sugar was present, the new sport was better nourished and outgrew the unaltered cells. Lewis's evidence points in this direction. He grew a different strain



of *Bact. coli mutabile* on a sugarfree agar, and found that some of the colonies, about 1 of 500,000, were capable of fermenting lactose. The proof is not absolute because no test is possible without bringing bacteria in contact with the sugar, and then, adaptation may occur. Sherman's experiments with the variation of Streptococci also seem to indicate a rather easy and spontaneous acquisition and loss of ability to ferment certain sugars.

This same logic has been employed to mutations produced by climatic changes through geological causes. Mutations may happen regularly, and independently of climate or environment, and only when the environment changes, may the mutant outgrow the original form.

For the study of chemical (and physical) factors in evolution, bacteria have decided advantages, but also great disadvantages, and it will depend upon the purpose of the experiment whether or not bacteria can be used.

While the geneticist works with hundreds and thousands, rarely with millions of individuals, the bacteriologist finds many million cells in each cc of his culture medium, and one liter of culture frequently contains  $10^{12}$  individuals. Such a population exceeding the human population of the world can be produced in 24 hours.

There are disadvantages in such large numbers. Only very small samples of such population can really be studied. The observation of lethal mutations would be absolutely impossible. A mutant which grows more slowly than the average is soon completely crowded out and will never be observed. A mutant which grows more rapidly than the rest will soon crowd out the original species.

A very extensive literature relates of such changes, morphological as well as physiological, and often the changes are so that the mutant represents not only a different species, but a different genus, and occasionally, even a different family, *e.g.*, the permanently irreversible asporogenous variety of *Bacillus anthracis* would, if found outside of an animal, have to be classified under *Bacteria-*

*ceae*, not *Bacillaceae*. This mutation can be brought about by continued cultivation on glycerol agar. Other striking changes are produced by cultivation on media with dyes, such as methyl green or malachite green, or with disinfectants like phenol or trichloroacetic acid. *Bacterium coli* has thus been changed to a bacterium which can no more form gas from sugar, and it would never be recognized as a variant of *B. coli* if isolated from water or milk.

This list of mutations could be extended over many pages. Most of these changes enforced by severe chemical treatment represent losses of properties, *i.e.*, destruction of genes rather than syntheses. They form a great contrast against the acquisition of new fermenting properties. By a combined loss of some properties and acquisition of others, the bewildering variety of all possible combinations of properties which makes taxonomy of bacteria next to impossible, is easily explainable.

This discussion does not include the much discussed changes from "smooth" to "rough" and "mucoid" and "G" forms of bacteria which are of great practical importance, but probably do not belong in this discussion. The author agrees with Hadley (1937) that we are dealing here with different manifestations of morphology and physiology whose range is not known in its entirety, but does not affect hereditary units.

#### SUMMARY

The object of this paper was to point out that evolution depends to a large extent on the formation of new kinds of hereditary units, *e.g.*, genes. Such formation can be caused only by a chemical reaction, and therefore must follow chemical laws. While the actual reagents involved are entirely unknown, some general laws can be applied successfully, and seem to justify the following statements:

The frequency of the creation of a new hereditary unit in any given species is proportional to the number of individuals born per year. This is borne out by the evidence that of the plants of North America, the rare families have

few species, and the common families have many species.

The frequency of the creation of a new hereditary unit in any given species is greater in warmer climates because chemical reactions proceed more rapidly at higher temperatures. This is borne out by the evidence that among reptiles, there are about eight times as many species in tropical climates as in moderate climates, while with mammals whose temperature is constant, the ratio is only 2:1. The number of species of plants also increases with the temperature of the country.

This evidence also indicates that the formation of new hereditary units is not caused by cosmic rays or any other kind of radiation, because the rate of reactions caused by radiation is independent of the temperature, and statistical evidence shows a dependence.

Polyploidy is not caused by chemical reactions, and it shows no relation to the number of species existing, nor does it increase with increasing temperature.

A change of environment is likely to affect cell chemistry, and to induce new reactions which might lead to the formation of new hereditary units. The frequency of such reactions should be proportional to the frequency of environmental changes. This is largely a question of size. Some microscopic organisms may change their environment daily on an enormous scale, *e.g.*, intestinal and soil bacteria. Variation in bacteria is so common that in several groups, species definitions are absolutely arbitrary because all shades of intermediate forms between any two "established" species have been described.

While the attempt has been made to collect for each point some convincing material, the author realizes that much more material must be treated statistically to prove the suggestions made in this paper. The author invites biologists to do this, since he expects to limit his own studies to bacteria.

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# SEX RATIOS AND TWIN PRODUCING KINDREDS

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## INTRODUCTION

It seems to have been well established through the work of Davenport (1920a, b, c, 1927), Curtius (1927), Dahlberg (1926, 1930), Greulich (1934), Hamlett (1935), Fisher (1928), von Verschuer (1932), Danforth (1916) and many others that the tendency to produce twins is of a hereditary nature, although there seems to be some difference of opinion concerning the nature and extent of this hereditary influence. On the one hand, Weinberg (1901, 1909, 1927), Bonnevie (1919, 1926), Eckert (1928) and Wehefritz (1925) seem to have evidence that indicates that only the dizygotic type is hereditary and that the monozygotic type occurs spontaneously in all cases. On the other hand, Davenport, Curtius, Dahlberg, Fisher, Greulich and others have presented evidence that clearly demonstrates a hereditary basis for both types of twinning. Curtius (1927) and Dahlberg (1930) conclude from their studies that both monozygotic and dizygotic twins occur in the same families and often in the same fraternities, while Bonnevie (1926) and Greulich (1934) conclude from their studies that, in the words of Greulich, "dizygotic and monozygotic twinning are not different expressions of the same twinning tendency, but on the contrary, seem to be phenomena quite distinct from each other."

Some further information that seems to bear on this general subject has developed from an attempt to separate kindreds<sup>1</sup> into groups on the basis of the type of twin represented in each. To this end, it was planned to make the

<sup>1</sup> A *kindred* is defined as a group of individuals each of whom is related in some way, either by blood relationship or by marriage, to every other member of the group and, in so far as is or can be known at any given time from the available records, to no other person.

separation in accordance with the fact that all kindreds which possessed one or more pairs of unlike sex twins would *certainly* possess factors for the determination of fraternal twinning. If the factors for the production of the two types of twins were entirely independent of each other, one would expect, in view of the low general incidence of .3912 per cent. for monozygotic twinning and of .7587 per cent. for dizygotic twinning, according to the figures of Greulich (1934), that there would occur only a very occasional instance when the factors for the production of identical and those for the production of fraternal twinning would occur coincidently in any one kindred. It seemed therefore that one might expect to be able to separate a given group of kindreds into two groups, one of which would represent practically all the occurrences of the factors for the production of fraternal twins, while the other would represent practically all the occurrences of the factors for the production of identical twins.

#### MATERIAL AND ANALYSIS

The studies were conducted upon all the kindreds containing 25 or more individuals which were filed under Trait 053 in the "A" File of the Eugenics Record Office. It was believed that kindreds of such size would provide kinship data of such quantity as would allow of further study, whereas smaller kindreds would have so few of such data as to make analysis very difficult and complex, if not impossible. Aside from this single consideration, the data of the archives were left unselected. It seemed that such an unselected group could be considered to represent a random collection of identical and fraternal twins in their typical and various relationships.

There were, in all, 85 kindreds of this size, and these contained a total of 919 twins. Of these twins, 26 pair were of unknown sex, 339 pair were of unlike sex, while 554 were of like sex. According to the difference method of Weinberg (1901, 1909) for calculating the number of identical and fraternal twins, and ignoring the 26 twins

of unknown sex, one may determine that there are  $893 - 678 = 215$  identical twin pairs in the population. This gives 24.1 per cent. identical to 75.9 per cent. fraternal twins. Dahlberg (1926, pp. 12-21) gives a detailed description of the history of the development and evaluation of the difference method, both before and after Weinberg's description (1901).

It is, of course, impossible to determine the effect that the twins of unknown sex would have upon the calculated frequency of identical twinning if the sexes could be known, but it is certain that though the proportion of identical twinning is slightly lower than the value of 26 per cent. cited by Newman (1937) based on data from a twin population of 717,907 twins provided by J. B. Nichols, and similar values provided by numerous other workers, it is still of sufficient magnitude to indicate the presence of a rather definite proportion of identical twins in this population. Eight triplets also occurred in the group, 2 of like sex, 5 of unlike sex, and 1 of unknown sex.

Further analysis of this group has shown that, among the 85 kindreds used in the study, some of the twins were specifically described with sufficient detail to enable one to determine whether twins (of like sex) were identical or fraternal in 14 of these kindreds. Of such descriptions, however, 22 pair were represented, of which 14 were identical and 8 fraternal. On the basis of the 75.9 per cent. fraternal twins and 24.1 per cent. identical twins found as described for the population as a whole, one would expect approximately 25 identical to 37 fraternal like sex twins, and, although the greater interest that exists in identical twins may be expected to result in a more frequent description of the occurrences of such twins, one may nevertheless regard the ratio of 14 identical to 8 fraternal twins as being a good indication that there is no unusual absence of identical twins in this population as a whole. In these characteristics, this group compares favorably with the groups that formed the bases for the earlier studies.

Since, however, it seemed that if the processes which underlay the production of these two types of twins were such as to render the causes of their production entirely independent, then one might expect that the coincident chance occurrence of both types of twinning, in view of the low general incidence of twinning in the general population of 1.15 per cent. according to Greulich (1934) as rendered in effect even lower by the high familial incidence of the trait as demonstrated by Davenport (1920a, b, c, 1927), Greulich (1934) and several others, would be very rare indeed. This postulated rarity of the expected occurrence of such coincidences led to a tentative working conclusion that it should be possible to separate the identical from the fraternal twins by making a selection upon the basis of the presence or absence of twins of unlike sex within a kindred. All kindreds which contain one or more twins of unlike sex would *positively* possess the factors for the production of fraternal twins, while, in the light of the foregoing, only in very rare instances would they contain factors for the production of identical twins. On the other hand, though the kindreds which contain only like sex twins might contain factors for the production of fraternal twins according to the laws of chance distribution, it would be expected that, by process of elimination, by far the larger proportion of such kindreds would contain only the factors for the production of identical twinning. The cases of coincident occurrence of factors for the production of both types of twins would be just as rare as in the group of kindreds where unlike sex twins occur.

On this basis, one would expect that the resultant distribution of twins would be such that practically all fraternal twins would occur in kindreds which contained one or more pairs of unlike sex twins, and that practically all the identical twins would occur in kindreds which contained only like sex twins. In view of the earlier determination, one would expect that approximately 24 per cent. of the total number of twins of the group, or 215 twins, would occur in kindreds which contain only like



sex twins, while approximately 76 per cent. of the total, or 678 twins, would occur in kindreds that contain one or more unlike sex twins.

The separation was made on this basis, and, quite contrary to these expectations, it was found that but 6.05 per cent., or 54 pair of twins, occurred in kindreds which contained only like sex twins, while 93.95 per cent., or 839 pair, occurred in kindreds that contained one or more pairs of unlike sex twins.

It might be reasoned that this condition was due to a failure in the reporting of all the cases of births of unlike sex twins caused by a lessened degree of interest in such twins, but this explanation could not possibly account for the *small* number of kindreds where only twins of like sex occur, while more accurate recording might even reduce the number of such kindreds. It is obvious, too, that it is not the relative numbers, but rather the relative *distribution* of the unlike sex twins that produces these conditions, and one would, therefore, not expect it to be due to a selective sexual selection, either in the form of a differential intrauterine mortality or in the form of sex reversals and intersexuality produced by a fusion of the circulatory systems in unlike sex twins. It is, indeed, difficult to conceive of a process whereby unlike sex twins could develop originally from identical or fraternal like sex twins, and the reverse process, though, of course, feasible, could not possibly effect the redistribution of the unlike sex twins required by the foregoing expectations.

It is, however, possible, though it seemed very improbable, that the condition could be produced by the simultaneous chance occurrence of the two types of twinning in a few of the large kindreds produced by a fusion by marriage of an original identical twin producing kindred with an original fraternal twin producing kindred. This might, in one such fusion, involve kindreds of sizes sufficient to account for the entire disturbance in the expected distribution among kindreds of like and unlike sex twins. In such a case, however, it would be expected that the

*entire* disturbance would be confined to but one, or at most, to but two very large kindreds, and that it should be possible readily to discern the two subgroups within the kindreds. As is shown in the following analysis, it has not been possible to do this.

Of the kindreds that formed the material for this study, the five largest contained 120, 93, 49, 35 and 33 twins, respectively. Five contained between 20 and 30 twins, while nine contained between 10 and 20, 24 contained between 6 and 9, 14 contained 5, and 28 contained between 1 and 4. The five largest of these kindreds were examined in order to determine if the distribution of the twins could be produced in the way already outlined.

These kindreds contained a total of 330 twins, or 37 per cent. of all the twins considered in the study, and of these, 125 were of unlike sex, 102 were male twins, and 103 were female twins. This would give an excess of like sex twins of  $103 + 102 - 125 = 80$ , or very close to 37.2 per cent. of the 215 excess like sex twins in the total population. Certainly, there is no sufficient massing of the like sex twins in any of these larger kindreds to account for the distribution of the twin types found.

Nor is any massing apparent in any of the other kindreds used in this study, as may be seen from a study of Table I, where the number of male twins, the number of female twins and the number of unlike sex twins are presented for each of the kindreds considered. This table is presented in five sections. In the first are grouped the 43 kindreds which have more like than unlike sex twins, in the second, the 14 kindreds having no unlike sex twins, in the third, the 14 kindreds having more unlike than like sex twins, in the fourth, the 10 kindreds having equal numbers of like and unlike sex twins, and in the fifth, the 4 kindreds having only unlike sex twins. Comparison of these groups shows that a total of 682 twins, or approximately 76.5 per cent. of all the twins, occurred in kindreds where there are more like than unlike sex twins. It is true, to be sure, that a large portion of these twins occur

TABLE I

RELATIVE OCCURRENCE OF THE TWIN TYPES IN THE KINDREDS HAVING MORE THAN TWENTY-FIVE INDIVIDUALS, ARRANGED IN FIVE GROUPS

Group I. More like than unlike sex twins				
Kindred reference	Number of twins			Total
	Like sex		Unlike sex	
	Male	Female		
39 .....	4	6	5	15
40 .....	2	3	2	7
42- 43 .....	2	1	2	5
73 .....	5	2	5	12
80 .....	2	3	2	7
81 .....	3	2	1	6
82 .....	2	4	2	8
83 .....	1	2	2	5
84- 85 .....	1	1	1	3
86 .....	2	2	1	5
97- 98 .....	1	5	1	7
101 .....	1	3	1	5
102 .....	1	2	1	4
106-110 .....	3		2	5
120-121 .....	4	2	1	7
132-133 .....	5	4	6	15
137-138 .....	2		1	3
140 .....	32	38	50	120
165-173 .....	4	6	5	15
184 .....	1	3	1	5
189-191 .....	5	3	1	9
197 .....	1	5	1	7
210 .....	2	3	1	6
230 .....	10	13	10	33
373-374 .....	3	1	1	5
407-414 .....	7	4	5	16
415-424 .....	4	4	6	14
425-432 .....	10	5	12	27
433-439 .....	7	9	12	28
460-464 .....	3	1	2	6
465-468 .....	3	1	2	6
469-475 .....	7	5	10	22
476-483 .....	14	7	14	35
484-487 .....	7	4	10	21
493-506 .....	18	12	19	49
507-511 .....	3	7	5	15
512-536 .....	28	33	32	93
538 .....	1	1	1	3
539-542 .....	2	3	4	9
700-701 .....	1	3	1	5
702 .....	1	4	1	6
818-820 .....		2	1	3
835-837 .....		3	2	5
Total 43 .....	215	222	245	682

in the larger kindreds of the whole group, but even if one consider only the kindreds that have less than 10 twins, he finds that there are, in this group, 152 twins, or 48 per cent. of all the twins that occur in such kindreds. By confining consideration to such kindreds, sixteen kindreds representing 530 twins are excluded from the group having more like than unlike sex twins, while a total of but 3 kindreds representing but 48 twins are excluded from the remaining four groups.

TABLE I—(Continued)

Group II. Only like sex twins				
Kindred reference	Number of twins			Total
	Like sex		Unlike sex	
	Male	Female		
75 .....	6	4		10
99 .....		2		2
105 .....	2	4		6
250-274 .....		1		1
275-278 .....	5	2		7
356-357 .....	3	1		4
381-382 .....		2		2
488-492 .....		3		3
638 .....		3		3
641 .....		2		2
643 .....	1	1		2
646 .....	1	2		3
659 .....	4			4
797-798 .....	4	1		5
Total 14 .....	26	28		54
Group III. More unlike than like sex twins				
1 .....		1	3	4
76-77 .....		1	2	3
78-79 .....	3		4	7
88 .....		1	2	3
95-96 .....	1		4	5
139 .....	2	1	6	9
141-142 .....	1	2	6	9
245 .....		1	3	4
371-372 .....	1	1	3	5
440-451 .....	9	3	14	26
636-637 .....	2		3	5
725-727 .....		2	3	5
791-793 .....	1	1	5	7
770-771 .....	1		3	4
Total 14 .....	21	14	61	96
Group IV. Equal number of like and unlike sex twins				
89-94 .....	1		1	2
131 .....	2	2	4	8
279-283 .....	1	2	3	6
452-459 .....	2	4	6	12
537 .....		1	1	2
626-629 .....	1	1	2	4
650-651 .....	1	2	3	6
653 .....	2	1	3	6
740-741 .....	3		3	6
759-760 .....	1	1	2	4
Total 10 .....	14	14	28	56
Group V. Only unlike sex twins				
198 .....			1	1
639 .....			2	2
658 .....			1	1
784-785 .....			1	1
Total 4 .....			5	5

The distribution of the twin types in the retained kindreds is presented in Table II, where the total number of kindreds, the total number of twins, the number of male, female and unlike sex twins corresponding to each twin composition of the kindreds is presented. In a group of kindreds of sizes varying symmetrically about an average

TABLE II

OCCURRENCE OF THE TWIN TYPES AMONG THE FIVE KINDRED SUBGROUPS IN THE GENERAL GROUP OF KINDREDS WHICH HAVE LESS THAN TEN PAIRS OF TWINS

Twin composition of kindred subgroup	Number of kindreds	Number of twins			Total
		Like sex		Unlike sex	
		Male	Female		
More like than unlike sex twins . . . . .	27	50	63	39	152
Only like sex twins . . . . .	13	20	24	..	44
More unlike than like sex twins . . . . .	13	12	11	47	70
Equal number like and un- like sex . . . . .	9	12	10	22	44
Only unlike sex twins . . . . .	4	..	..	5	5
Total . . . . .	66	94	108	113	315

of approximately 4.75 twins per kindred, as may be seen in Fig. 1, one would expect, on the basis of chance distribution of fraternal twin types, an essentially equal number of twins to occur in kindreds where more like than

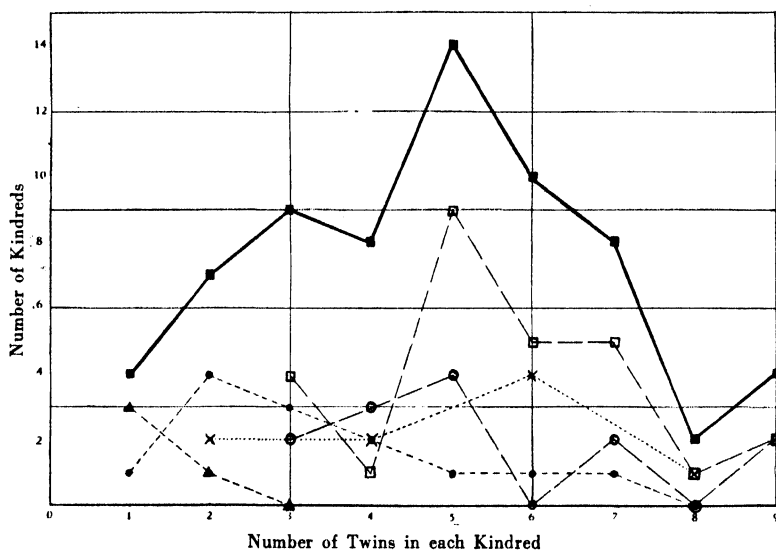


FIG. 1. Graph showing frequency distribution of kindreds on the basis of number of twins that appear in each. The distribution of all kindreds is shown by solid squares, while the five subdivisions of this total group are shown by circles and squares for those having more unlike than like, and those having more like than unlike sex twins respectively. Solid triangles and circles represent those having only unlike, and those having only like sex twins respectively, while crosses represent those having equal numbers of like and unlike sex twins.

unlike sex twins occur and in kindreds where more unlike than like sex twins occur, and an essentially equal number of twins to occur in kindreds where only twins of unlike sex occur and in kindreds where only twins of like sex occur. The excess of twins in the latter group, or  $44 - 5 = 39$  twins, should represent approximately the number of identical twins in the total population. These 39 twins represent, however, but 12.5 per cent. of the total number of twins, although the excess of like sex, and so, presumably, of identical twins is  $315 - 226 = 89$ , or 28.4 per cent. On the other hand, in the other two groups of kindreds, there is a total of 86 twins of unlike sex. These call for an equal number of like sex twins, which leaves  $222 - 2(86) = 50$  excess like sex twins in kindreds where unlike sex twins occur.

It is therefore obvious that the unexpected distribution of the twin producing kindreds on the basis of presence or absence of unlike sex twins is *not* produced by an occasional simultaneous chance occurrence of the two types of twinning in the same kindred. Instead, it is shown that the unexpected distribution occurs rather widely and in a large majority of the kindreds, irrespective of their size.

On the other hand, however, the condition is not merely that of a natural random scatter of identical twins, as can be readily shown from already available statistical data. Thus, taking the figure of 1.15 per cent. of the U. S. Census Report for the incidence of the occurrence of twins in the general population, and using the figure of 24 per cent. to represent the portion of these that are believed to be of the identical or uniovular type, then, if one assume that the identical twins occur entirely independently of the fraternal twins, it would follow that the random distribution of such twins would have an incidence of occurrence of approximately  $.24(1.15) = .276$  per cent., or about 276 in 100,000 births. One would expect, on this basis, about 2.465 identical twins to occur in the group of 893 twin births, and one obtains essentially the same results if he assumes that identical twinning is hereditary. In

such a case, the identical twins would occur in groups of two or more, but the incidence for the occurrence of the groups would be correspondingly less. Certainly, chance coincidence of small groups of identical and fraternal twins in the same kindreds could not occur frequently enough to account for the distributions that have been found.

It seems from this, then, that one is forced to conclude that the factors for the determination of identical twinning and those for the determination of fraternal twinning are *associated* in some way. Final conclusive proof of the occurrence of this association is provided by a further study of the 22 pair of like sex twins that occur in the 85 kindreds of this study for which *specific* descriptions regarding their type are provided. Of these, but four pair of identical twins occur in kindreds in which no fraternal twins or twins of unlike sex occur, while in one kindred in which only like sex twins appear, two pair of fraternal twins occur. On the other hand, specifically described identical twins occur in seven kindreds representing 10 pair of twins in which twins of unlike sex occur. In two of these kindreds representing three pair of identical twins, three pair of like sex fraternal twins are also specifically described. Three like sex fraternal twins with no identical twins are described in three kindreds in which unlike sex twins also occur. These conditions are shown in Table III.

And final confirmatory evidence for the association between the factors for the production of identical twins and those for the production of fraternal twins comes from a study of the smaller kindreds filed in the 053 series of the "A" File of the Eugenics Record Office Archives. In this group, there occurred 108 kindreds which contained specifically described identical or like sex fraternal twins. Forty-nine of these contained 2 or more twins, and 24 of these contained one or more identical twins. Of these 24 kindreds, 15 positive cases representing 62 per cent. of these kindreds presented an occurrence of identical twins

TABLE III

OCCURRENCE OF THE SPECIFICALLY DESCRIBED IDENTICAL AND LIKE SEX FRATERNAL TWINS AMONG THE KINDREDS HAVING MORE THAN TWENTY-FIVE INDIVIDUALS, IN RELATION TO EACH OTHER AND TO THE UNLIKE SEX TWINS OF THE GROUP

Kindred reference	Specifically described twins				Undescribed twins			Total
	Identical		Fraternal		Like sex		Unlike sex	
	Male	Female	Male	Female	Male	Female		
73.....	2				3	2	5	12
131.....	1				1	2	4	8
106-110.....	1		2				2	5
120-121.....	2		1		1	2	1	7
373-374.....	2				1	1	1	5
818-820.....		1				1	1	3
835.....		1				2	2	5
275-278.....	1	1			4	1		7
381-382.....		1				1		2
797-798.....		1			4			5
356-357.....			2		1	1		4
245.....				1			3	4
740-741.....			1		2		3	6
759-760.....			1			1	2	4
Total 14	9	5	7	1	17	14	24	77

TABLE IV

OCCURRENCE OF SPECIFICALLY DESCRIBED IDENTICAL TWINS IN KINDREDS WHICH, IN THE FIRST SECTION, ALSO HAVE ONE OR MORE PAIRS OF LIKE SEX FRATERNAL OR UNLIKE SEX TWINS, AND, IN THE OTHER SECTION, HAVE ONLY IDENTICAL OR UNDESCRIBED LIKE SEX TWINS

Kindred reference  Section I	Specifically described twins				Undescribed twins			Total
	Identical		Fraternal		Like sex		Unlike sex	
	Male	Female	Male	Female	Male	Female		
19.....		1		1			1	3
112-115.....		1		1			1	3
216-217.....		1					1	2
375-376.....		1				1	1	3
379.....	1						1	2
709-710.....	1					1	2	4
711-712.....		1					1	2
739.....		1			2		1	4
754-755.....	1				1		1	3
765.....		1			1		2	4
782-783.....	1				2	1	2	6
795-796.....	1					1	1	3
806-807.....	1				1		1	3
815-816.....	1						3	4
834.....		1					1	2
Total 15	7	8	0	2	7	4	20	48
Section II								
181-183.....		1			1			2
714-716.....		1			1			2
732.....		1				1		2
733-734.....	1				1	3		5
762-763.....	2				1			3
788-789.....	1					1		2
808.....	1					1		2
812.....		1			3			4
827-828.....	1				1			2
Total 9	6	4	0	0	8	6	0	24



and like sex fraternal or unlike sex twins in the same kindred and two of these cases showed the occurrence of identical, like sex fraternal and unlike sex twins in the same kindred. On the other hand, there occurred but 9 cases where neither like sex fraternal nor unlike sex twins appeared in kindreds containing one or more identical twins. Thus it is evident that identical twins do positively occur in kindreds where like sex fraternal and unlike sex twins similarly occur and that they occur in such kindreds just as frequently and often much *more frequently* than they do in kindreds where fraternal and unlike sex twins do not occur. These conditions are shown in Table IV.

#### DISCUSSION

Monozygotic twins, by definition, are produced from a single zygote which, at some time during its development, divides more or less completely into two (or more) centers of active growth and differentiation. This separation is not always complete, as has been shown by Stockard (1921) for the fish, and by the numerous cases of Janus, Siamese and other twin monsters of Morrill (1919), Wilder (1904, 1908), Fisher (1866) and many others, that have occurred in man. There is still some lack of agreement concerning the exact causes of this condition, but if the work of Stockard on the fish, and of Spemann (1903) and Hey (1911) on Triton, has any transference value, it would seem that Janus, Siamese and other types of conjoined twins that occur in man are produced by the simultaneous occurrence of two separate and distinct by nearly lying centers of gastrulation in the embryonic disc or blastoderm.

As development proceeds, these two centers would encroach upon each other. Certain of the most closely adjacent portions would fuse, while the remaining portions would continue an independent development. Depending upon the distance between the two centers of gastrulation would depend the degree of fusion between the two "individuals" of the twin monster, until finally

the intervening distance would become sufficient to insure the continued independent integrity of the developments from each center of gastrulation. Newman and Patterson (1910) and Patterson (1913) have described such a condition in the armadillo, while Kaestner (1898, 1907) and several others have described it in the chick, Kopsch (1895) in the European lizard, McIntosh (1868) and Reese (1911) in the cat, Carey (1917) in the pig, Bishop (1908) in the reptiles and Wetzel (1900) in the snake.

It is probable, according to Abrams (1921) and others, that all twin monsters and the most closely located, but wholly independent, twins would have but a single amnion, as in the case of the armadillo, whereas twins arising from still more distantly located centers of gastrulation might have various degrees of incompletely separated amnia until finally the condition of two distinct amnia might occur. It has been found that monoamniotic twins occur in approximately 2.11 and 2.4 per cent. of the cases of uniovular twins, according to Resinelli and Alfieri, respectively, as cited by Jeannin (1906).

The causes of this condition are, according to Stockard (1921), attributable to environmental conditions, but this view as the primary cause of twinning has been questioned by Riddle (1923), who found in his studies of identical twinning in pigeons and doves that the factors for the production of such twins operate at a stage much earlier than that of gastrulation. He was, moreover, unable to induce doubling in any of 2,500 normal eggs by means of subjecting them to various environments during or from 5 to 8 hours prior to the time of gastrulation.

It has, however, been shown that not all types of monozygotic twinning are produced by the simultaneous establishment of two centers of gastrulation in the blastoderm, for it has been shown by Assheton (1898) (1908) in the sheep and ferret, by Wetzel (1900) in the snake, by Corner (1922) and Streeter (1924) in the pig and by Streeter (1919) and Arey (1922) in man that twinning may also occur by the simultaneous development of two inner cell

masses in mammals or by the development of two or more separate and distinct blastoderms in reptiles. Corner (1922) attempts to correlate these two general types of twinning by suggesting that there might be intermediate and unclassified stages. The two inner cell masses of the latter type of twinning may be separated by a varying distance, and may, on the one extreme, come to lie so closely together that the two blastoderms of the two inner cell masses fuse into one, but each develops its embryo independently as though the fusion had not occurred.

And finally, it seems, if the work of Driesch (1892) with the sea urchin, Wilson (1893) and Cerfontaine (1906-1907) with *Amphioxus*, Spemann (1914) and Rudd (1925) with *Triton*, Morgan (1893) with the fish, McClendon (1910), Schultz (1894), Morgan (1895) with the frog, and many others, in which it was found that two embryos, or twins, could be produced by the separation of the two primary blastomeres, has transference value when applied to man, then a third type of monozygotic twinning might be expected. Twins of this type, though monozygotic, would be expected to have separate chorions and placentae. The frequent difference noted by Danforth (1916), Curtius (1928, 1930), Lassen (1931), and several others, between the number of monozygotic twins when determined by the "difference method" and by the "similarity method," on the one hand, and by the "birth membrane method," on the other, might in part be considered to represent monozygotic twins of this type.

Dizygotic twins, by definition, are produced from two zygotes that arise and become successfully implanted during the same oestral cycle. It has been shown by Arnold (1921) and Davenport (1920) that probably between 5 and 10 per cent. of all ovulations are double. Ova, therefore, seem to occur in an excess of about 4 to 9 times the frequency of twin births. Davenport considers this to indicate that the limiting factor for the production of dizygotic twins lies elsewhere than in the number of ova available for fertilization during a single ovarian cycle.

These limiting factors may reside in some phase of the fertilization process, in lethal factors of some sort occurring in the zygote, in the implantation mechanism, or in subsequent early incompatibility between mother and embryo. Davenport seems to emphasize the importance of lethal factors introduced into the zygote by the paternal or the maternal line.<sup>2</sup>

The relation of these varying modes for the development of monozygotic and dizygotic twins to fundamental, underlying genetic factors may be of several sorts. It has been shown by Morgan, Sturtevant, Muller and Bridges (1923, pp. 32-34) that two or more phenotypic characters may always occur together. Such cases are considered to be so closely linked that they have a cross-over value of zero, hence are considered each to have exactly the same gene locus, or, more simply, to be two manifestations in different portions of the phenotype of one and the same gene. There could be no more reason for suspecting, *a priori*, that such phenotypic characters might be thus related than for suspecting, *a priori*, that two phenotypic characters might be genetically linked. Such occurrences are detected entirely by genetic procedures, and proof of their occurrences rests entirely on such determinations.

<sup>2</sup> A third type of twinning has been suggested by Danforth (1916), Fisher (1919) and Curtius (1927), and been regarded favorably by several others. The general idea involves the formation of two cells, each containing a female pronucleus from the original ovum. This may occur either, as Danforth suggests, by a precocious division caused by the penetration of the egg by the spermatozoon so that the male pronucleus is able to unite with only one half of the original egg nucleus, leaving the other half free for fertilization by a second spermatozoon, or as Curtius suggests, by the fertilization by separate spermatozoa of both the definitive ovum and its unusually large second polar body. In view of the lack of any embryological evidence concerning the occurrence of this type of twinning, one is forced to regard it skeptically, despite the fact that it would seem to explain many of the difficulties of Fisher (1919) and others who, from statistical studies on twins classified by means of the "similarities method," find need for a type of twin that is genetically intermediate between the completely dizygotic twin with its four different pronuclei and the monozygotic twin with only two different pronuclei.

It may be, however, that these types of twinning are produced by one of these two processes. If subsequent studies show that all types do always occur together in the same kindred, then depending upon the nature of the key process concerned respectively in the production of each type, and the results of genetic studies in order to test linkage, it might be found that these types of twinning are either the expression of several different and entirely distinct phenotypic manifestations of the same gene, or are the phenotypic manifestations of several genes which, however, are linked with a to-be-determined degree of closeness.

On the other hand, it has been shown by Hoge (1915) and others that a gene may produce a phenotypic character or condition which of itself may underlie or lead to the production of numerous phenotypic characteristics, the origin of all of which, however, may be traced eventually to the one genetically determined phenotypical condition. *A priori*, one would expect to be able to detect such a condition by noting some sort of fundamental similarity among several phenotypical characteristics that seem to be related and also genetically determined. Intergradations between the two types might very conceivably occur.

From this point of view, it seems reasonable, on the basis of the foregoing discussion and on the basis of the observations presented in this article, to suggest that possibly a single genetic factor might be responsible for the production of a fundamental underlying phenotypic condition which might secondarily produce or manifest itself in a highly varied but always fundamentally similar manner. Such a character in these cases of twinning might be termed "*Weak Dominance*," and might be specifically defined as "a tendency, manifested throughout the early life of the zygote, for primary growing points to fail to adequately dominate the secondary and other potential growth areas, with the result that, frequently, two primary growing points arise where normally but one would occur."

In the case of monozygotic twinning of the type where the two blastomeres separate, this might take the form of a weakened dominance of the organism as a whole over its constituent parts. The work of F. R. Lillie (1902, 1906) on the differentiation of the egg of *Chaetopterus* with cleavage experimentally suppressed would indicate that what might be termed organism-as-a-whole forces are, in this case, independent of the cleavage mechanism. If such experiments have transference value, it might indicate that similar conditions might occur in man. On the other hand, the work of Herbst (1900), on raising eggs of echinoderms in calcium free sea-water would indicate that separation of the blastomeres is largely a process dependent upon environmental influences.

The manner of formation of the inner cell mass may also be one of dominance. It would seem from the observations of Assheton (1898), Corner (1922) and Streeter (1924) that at least more than one group of the cells of the morula can form an inner cell mass, or that the cohesive forces which ordinarily cause all the cells of the morula to remain together may occasionally be weakened so as to lead to a splitting whereby these inner cells form two separate and distinct masses, each of which undergoes subsequent independent development. In either of these two methods, a slight weakening of the normal dominance of one part over the other parts at a certain time may very readily lead to the establishment of two equally strong growth points instead of the one that normally occurs.

The importance of dominance by primary growth centers in the blastoderm at the time of gastrulation, and the frequent production of twinning when such dominance is disturbed at certain times by experimental methods has been extensively described by Stockard (1921). Any weakening of the dominance of the primary centers of gastrulation at this stage produced by hereditary factors might, likewise, very possibly, result in the establishment

of two centers of gastrulation instead of one as occurs normally.

And finally, in the case of the dizygotic twinning, from this point of view, the underlying cause might reside in a weakening of the dominance of the zygote at the time of implantation in such a way that the uterus would remain receptive for a longer time than normally. In such a case, it might frequently happen that a second zygote might become well implanted before the uterus would be rendered utterly unreceptive. Certainly, it has been shown by Leo Loeb (1923), Corner (1921), Courrier and Kehl (1930), Knaus (1930), Asdell (1928), Pratt (1927) and several others that the effect of the implantation of the zygote is to cause the retention of the corpus luteum, which in turn secretes hormonal substances which render the uterus retentive of zygotes already implanted, but unreceptive to others.

The nature of the dominance mechanism is still subject to some question, but the work of Jacques Loeb (1924) on *Bryophyllum* and of Spemann (1936) on "organizing substances" in the dorsal lip of the blastopore indicate that definite substances are involved. It is conceivable that the amounts of such substances might vary and that the amounts might be determined by underlying hereditary factors.

The association between the occurrences of identical and fraternal twins demonstrated in this paper confirms the observations of Curtius (1927) and Dahlberg (1930). Curtius, relying solely upon identifications on the basis of physical similarities, notes cases of the occurrence of like sex fraternal and identical twins in the same families and sometimes in the same fraternities, and cites in addition some cases of Battstrom (1914) and Hoehne (1920) of triplets, quadruplets and quintuplets in which some of the individuals are clearly identical and some clearly fraternal. His material, however, is somewhat limited.

Dahlberg (1930) provides some data obtained from mothers who had already given birth to one pair of twins.

*Not counting these twins*, he obtains a ratio of 48 like sex pairs to 24 unlike sex pairs from mothers who had previously produced an unlike sex pair of twins, and again not counting the first twins, obtained a ratio of 18 unlike to 38 like sex pairs from mothers who had previously produced a like sex pair of twins. It seems as though the exclusion of the first pairs of twins born to these mothers significantly reduces the value of the conclusions which he derives from these data.

Working with other forms, Green (1934) has explained certain genetic ratios which he obtained from crosses of mice on the basis of the occurrence of hereditary monozygotic twinning, while Danforth (1925) by selecting parents who had produced incompletely separated twin monsters was able to isolate strains of mice having a markedly augmented incidence for the production of such twins. The cases where monozygotic twins have been described in the pig by Corner (1922) and Streeter (1924) show that in that form identical and what would correspond to fraternal twinning occur together, though the origin of the material rendered impossible studies for the determination of the mode of inheritance of such twinning.

On the other hand, Bonnevie (1925) and Greulich (1934) have definitely concluded from their observations that dizygotic and monozygotic twinning are quite distinct from each other. Bonnevie's conclusion follows directly from her observations that only the dizygotic type of twinning is hereditary, but Dahlberg (1930) has computed the probable errors for her data and finds that the amount of her data concerning one-egg twinning is much too small to allow of reliable conclusions.

Greulich, basing his conclusions on a population of 312 individuals containing 94 monozygotic twins in 91 families of sizes from 1 to 10 births and on a population of 1,017 individuals containing 296 twins in 270 families of sizes from 1 to 10 births, concludes that "the parents of monozygotic twins differed from the parents of dizygotic twins



in their capacity for twin production: the latter had twins much more frequently than would be expected if twinning were determined by chance alone, while the former showed a frequency of twin births only very slightly higher than chance expectation." In view of the fact that he computed the incidence of monozygotic twins in the general population to be approximately 4 per 1,000, and obtained 3 per 221 individuals, and for dizygotic twins to be approximately 7.5 per 1,000, and obtained 26 per 747 individuals, it would seem that the sizes of the populations that he used for these determinations were much too small to justify his conclusions. It would be interesting to know the distribution of monozygotic and dizygotic twinning in his studies of the incidence of twinning among the sibs of twin-producing parents.

It has been shown in this article that the factors for the production of the two types of twinning occur together with a significantly high degree of regularity. The causes of this association may be any one or combination of several conceivable possible factors. Twin production is a very complex phenomenon that requires for its successful completion an optimal phase of a wide variety of fundamental and underlying, contributory conditions of the nature of high general gametic compatibility and vitality, anatomical suitability, nutritional and physiological vigor and generally favorable environmental conditions.

In so far, however, as twinning may be proved to rest upon a distinctly genetic basis, so then this demonstration of the association between the two types of twinning might be regarded as an indication of a genetic linkage of *some sort* between the factors for the production of identical twins and the factors for the production of fraternal twins. If further studies should prove that the determining factors are dependent upon genetically linked genes, then one would have good reason to expect to be able to discern the occurrence of autosomal linkage in these phenomena.

## SUMMARY

An association between the factors for the production of identical and those for the production of fraternal twinning has been shown to occur rather widely and in a significantly large majority of the kindreds, irrespective of their size. The occurrences of this condition have been shown both by means of the "difference method" of analysis for the ratios of like and unlike sex twins that occur in the same kindred, and by means of a detailed study of the occurrences of specifically described identical twins in kindreds which also contain specifically described like sex fraternal or unlike sex twins. It was demonstrated that identical twins do positively occur in such kindreds and that they occur in that way much more frequently than they do in any of the other kindreds. It has been shown conclusively that the chance coincidence of small groups of identical and fraternal twins in the same kindred certainly could not occur frequently enough to account for the distributions which have been found.

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# ORDER OF DIFFERENTIATION IN RELATION TO ORDER OF DETERMINATION IN GAMIC FEMALE APHIDS

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## INTRODUCTION

WHEN winged parthenogenetic female aphids in the gamic phase of the cycle are first reared in low temperatures and then changed to high temperatures, their successive offspring gradually change from gamic to parthenogenetic females (Shull, 1930a). Aphids intermediate between gamic and parthenogenetic are born during the transition. Studies on the composition of the intermediate aphids (Shull, 1930b, 1931, 1933) have shown that the first intermediates to appear have modified antennae and hind tibiae, later offspring showing changes in the reproductive system. In these modifications of the reproductive system the earliest offspring lose their colleterial glands and seminal receptacle, while later ones exhibit various changes in the ovarioles. The order of change is thus antennae and hind tibiae, then colleterial glands and seminal receptacle, then ovarioles.

A possible explanation of this order of change in the intermediates (Shull, 1930b), similar to that used by Goldschmidt (1927) to explain intersexes, is that only those structures in the embryo are affected that have not been determined at the time of the temperature change. Thus the first structures to be changed (antennae and hind tibiae) in a series of successive offspring would be those that are determined late, the last to be changed (ovarioles) in such a series would be those that are determined early. The order of embryonic determination thus would be ovarioles, then colleterial glands and seminal receptacle, then antennae and hind tibiae.

If determination of a structure leads to its differentiation soon thereafter, or if equal periods of time intervene

between determination and differentiation, the differentiation should likewise be (1) ovarioles, (2) colleterial glands and seminal receptacle, (3) antennae and hind tibiae. It is important to know whether this is the order. This paper gives the results of an investigation designed to determine the order and time of differentiation.

#### MATERIAL AND METHODS

The order of differentiation was established by the study of slide preparations of gametic embryos and first, second and third instar gametic females. Both parthenogenetic and gametic females were used for the study of time of development of the different structures. As all aphids with the exception of the stem mother are produced viviparously and parthenogenetically there is no externally observable event such as fertilization or egg deposition by which the beginning of development can be timed. It was observed, however, that parthenogenetic embryos about to be born contain within their ovarioles embryos of the next generation. The oldest of these embryos are well along in cleavage when the parent aphid is born. The development of the newly born aphid into an adult is paralleled by the development of the enclosed embryos so that the time elapsed from late cleavage to the development of any particular structure in the oldest embryo can be determined by fixing the parent aphid at a measured time after birth and examining the oldest enclosed embryo. To determine the total age of the oldest enclosed embryo at any particular time it is necessary to add to the above time the time elapsed between maturation of the parthenogenetic egg and late cleavage. It is impossible to determine the beginning of maturation without killing the egg and thus stopping development, hence the total time of development can not be computed for any one aphid. This can be done only by adding the time of development of the two different stages taken from two different aphids. As variation occurs in the rate of development in different individuals an accurate measure can be made only by

statistical handling of a great number of individuals. Sufficient specimens were not collected to do this, hence all references to time of development refer to time measured from late cleavage.

Measurements of time of embryonic development computed indirectly on the basis of rate of growth of a maturing aphid are subject to variation. Shull (1930a) has shown that the rate of postnatal development of aphids is modified by the prenatal conditions of light and temperature. By varying the light and temperature conditions applied to the parents he found that some parthenogenetic females matured at the mean age in days of  $8.0 \pm 0.10$ , while others did not mature until reaching a mean age in days of  $12.0 \pm 0.91$ . My own observations indicate that a variation in the rate of postnatal development occurs under ordinary laboratory conditions. Of 17 cases recorded one required 6 days to become adult while several required 9.5 days. The mean age in days of the 17 aphids was  $8.2 \pm .26$ . That this variation of postnatal development has an effect on the rate of development of the embryos contained within the growing aphids is shown by a comparison of a fourth instar aphid with an adult, both of which were 8 days old. The embryos in the adult were much more advanced in development than those in the fourth instar.

It is possible that the variation in rate of embryonic development caused by a variation in rate of postnatal development of the parent affects certain periods of embryonic development only and does not affect the total time of development, which thus may be the same for all aphids. A microscopic examination and comparison of embryos of the same age would show a difference in degree of development if such exists, but would give no indication whether these differences were equalized by the time of birth. It is possible that retardation of development at one stage would be compensated for by later increase in the rate of development and, conversely, that an early rapid rate of development would be equalized by a later slow rate.



The age of parthenogenetic aphids at the last molt varies in 17 observed cases from 6 days to 9.5 days. At the time of birth each of the 17 aphids contained one or more embryos undergoing cleavage, which at the time of the last molt would be almost completely developed and ready for birth. The age of the embryos would vary depending upon the age of the parent so that the oldest embryos contained in the aphid that matured at 6 days of age would be 6 days, while the embryos contained in the aphid that matured at 9.5 days would be 9.5 days old. If the total time of embryonic development is the same for all aphids the time between the last molt and the birth of the first young should be at least 3.5 days longer for the former aphid (6 days) than for the latter (9.5 days). Such a difference in time between the last molt and birth of the first young does not exist. The aphid that matured at 6 days of age gave birth to its first young 24 hours later, and one of the aphids that matured at 9.5 days likewise produced its first young 24 hours later. Thus the total time taken for embryonic development was 7 days in one case and 10.5 days in the other. One aphid that matured at 9.5 days produced its first offspring 46 hours after the last molt, thus making the total time of development for this embryo approximately 11.5 days.

#### ORDER AND TIME OF DIFFERENTIATION OF REPRODUCTIVE STRUCTURES

The first reproductive structures to appear in the embryo are the germ cells. No attempt was made to discover their exact origin, but by the time the blastoderm is formed about 24 hours after early cleavage the germ cells are evident as a rounded cluster of cells within the blastocoele (Fig. 1). At this time the blastocoele is being filled up with small corpuscles that later make up the symbiotic organ. These corpuscles are derived directly from a localized area on the ovariole containing the developing embryo. The germ cells lie at one side of the blastocoele, hence are not quite surrounded by the newly formed

symbiotic organ. This same spatial relationship between germ cells and symbiotic organ is continued throughout the life of the aphid. The germ cells increase in number as development proceeds and become arranged in clusters, each of which is surrounded by a thin membranous sac. Five days after cleavage the clusters of germ cells are recognizable as germaria in which the nurse cells and oogonia have become differentiated. By this time the body cavity is formed and the germaria are found ar-



FIG. 1. GC—Germ Cells; SO—Symbiotic Organ.

ranged in two groups one on each side of the cavity, the rest of which is filled with the symbiotic organ.

Ovarioles appear about 5 days after cleavage as outgrowths of the membranous sacs surrounding the germ cells. A short distance from the germaria the ovarioles of each group unite forming two parallel oviducts which extend to the posterior lateral tip of the abdomen. The end of each oviduct enlarges into a terminal ampulla, the base of which rests against the thickened ectoderm ventrad and laterad of the anus. Thus one terminal ampulla is right and the other is left of the ectoderm immediately ventrad of the anus. The oviducts develop on the sixth day after cleavage.

It is important to know at what time during development the ovarioles first exhibit their gamic nature. For at least 24 hours after the ovarioles are differentiated there is no visible difference between gamic and parthenogenetic types. Some time before birth (about 24 hours) parthenogenetic ovarioles receive eggs undergoing maturation. Ovarioles of gamic females of the same age are devoid of eggs and do not contain eggs until 4 or 5 days after birth. This lack of reproductive activity in embryonic gamic germaria and ovarioles in contrast to the production of eggs in the parthenogenetic females indicates that determination of both types has occurred within the embryo.

Gamic eggs probably begin maturation during the fourth instar. The evidence is not exact on this point, but none of the slides of third instar gamic females show maturing eggs, while all the adults show the eggs plainly. There is some doubt that the slides of fourth instar aphids are actually fourth instar, hence the evidence from these slides is omitted. The inability to state the exact time of maturation of gamic eggs is of no great importance at this time. The significant fact is that gamic eggs are produced after birth only. This point will be enlarged upon later.

The vagina is differentiated next as an invagination of the ectoderm ventrad of the anus and between the two terminal ampullae. The first indication of the development of the vagina is a thickening of the ectoderm between the terminal ampullae (Fig. 2). This occurs about the seventh day after cleavage or approximately 48 hours before birth. The invagination begins about 24 hours later and continues after birth.

The colleterial glands and seminal receptacle develop as outgrowths of the vagina during the second instar (2 or 3 days after birth and 5 or 6 days after differentiation of the ovarioles). One colleterial gland appears on each lateral side of the vagina while the seminal receptacle develops as an evagination of the dorsal vaginal wall (Fig. 3).

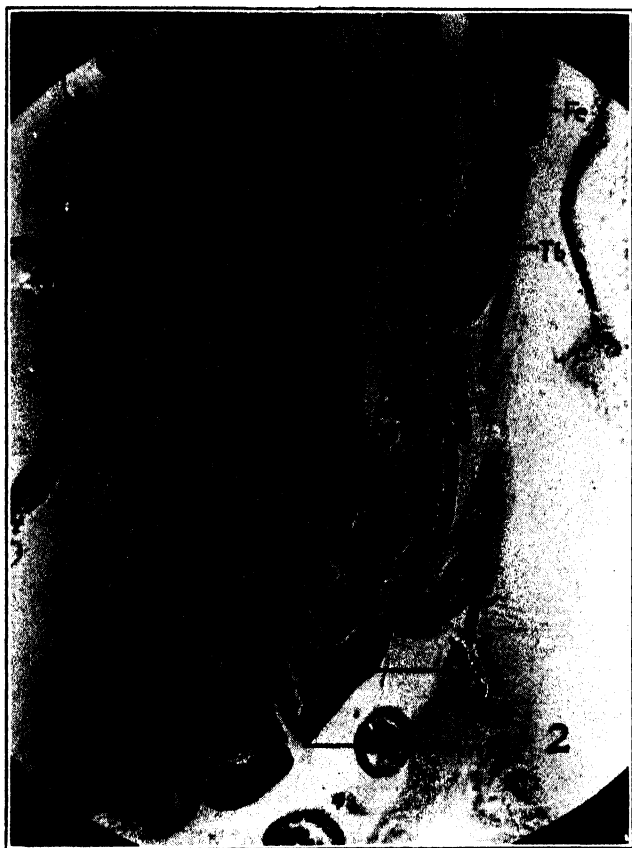


FIG. 2. An—Anus; Fe—Femur; Tb—Tibia; V P—Vaginal Primordium.

It has been shown by Shull (1930a) that determination of the colleterial glands and seminal receptacle occurs before birth. Hence the appearance of these structures 2 or 3 days after birth indicates a delay in differentiation. Evidence of an earlier differentiation within the embryonic period was sought by comparing the vaginas of gametic and parthenogenetic female embryos. Any character by means of which gametic and parthenogenetic female embryonic vaginas could be differentiated could be used in the case of the gametic female to predict the future appearance of the colleterial glands and seminal receptacle, and consequently the appearance of this vaginal character could be



FIG. 3. SR—Seminal Receptacle; Va—Vagina.

considered to be the first indication of the differentiation of the colleterial glands and seminal receptacle. No such character was found so it must be concluded that a delay in differentiation actually takes place.

#### DIFFERENTIATION OF HIND TIBIAE

The appendages begin development as outgrowths of the sides of the embryo on the fourth day after cleavage (Fig. 4). Twenty-four to 48 hours before birth they differentiate into femur, tibia, tarsus and claws. Fig. 2 shows a longitudinal section through the joint connecting the femur and tibia of the metathoracic leg shortly after segmentation of the appendage. Further development



FIG. 4. Ap—Appendage; G C—Germ Cells.

consists mainly of growth in size until at the time of the last molt ( $8.2 \pm .26$  days after birth) when the hind tibiae undergo their last change and develop gamic characters which consist of a swollen condition, dark coloration and sensoria (Fig. 5).

It is questionable which event in the development of the hind tibiae represents the most significant period in relation to the determination of the gamic nature of the appendage. Differentiation of the hind tibiae undoubtedly occurs at the time of the segmentation of the appendages. However, as there is no morphological difference between parthenogenetic and gamic female appendages at this time there is no reason for assuming that this event constitutes the differentiation of the gamic nature of the hind tibiae. The appearance of the adult gamic hind

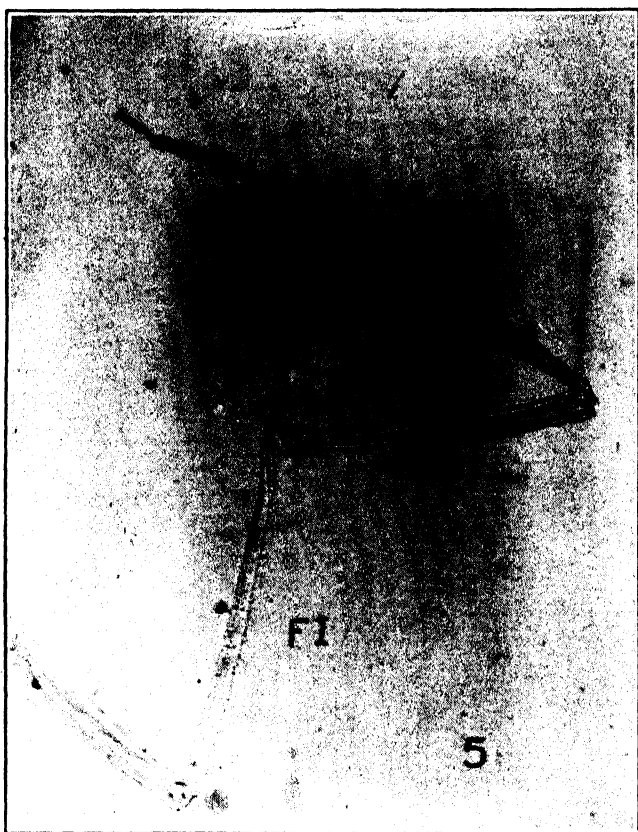


FIG. 5. Ad—Adult; FI—Fourth Instar.

tibial characters at the time of the last molt marks the first visible difference between gamic and parthenogenetic hind tibiae, consequently this event is assumed to be the differentiation of gamic hind tibiae. It is probable that some sort of physiological differentiation occurs before this, especially since determination occurs before birth, and that the gamic hind tibial characters simply represent the adult expression of the earlier differentiation. Despite this probability it is necessary to conclude on the basis of the visible evidence that differentiation does not occur until the last molt and, therefore, that differentiation is delayed about 8 days after determination has taken place.

ORDER OF DIFFERENTIATION COMPARED TO ORDER  
OF DETERMINATION

The order of differentiation is (1) ovarioles, which appear about the fifth day after cleavage, (2) colleterial glands and seminal receptacle, which appear two or three days after birth, (3) hind tibiae upon which gamic characters develop about eight days after birth. Proof that the order of differentiation is as stated is given in the illustrations. Fig. 5 shows a fourth instar and an adult hind tibia. The absence of swelling and sensoria on the fourth instar appendage is apparent. In Fig. 3 the seminal receptacle is shown in the early stage of its growth from the dorsal wall of the vagina. This figure is to be compared



FIG. 6. An—Anus; Od—Oviduct; Ov—Ovariole.



with Fig. 6, which shows no evidence of a vagina but which does show the presence of ovarioles and an oviduct. The evidence from intermediate aphids that determination occurs in the following order (1) ovarioles (2) colleterial glands and seminal receptacle (3) hind tibiae is thus supported by the order of differentiation.

#### DISCUSSION

Certain facts developing out of the study of differentiation indicate that the original hypothesis which explains the production of intermediate aphids on the basis of different times of determination for different structures may be partially correct only. The time of determination theory explains the production of intermediates by assuming that in any embryo the structures involved have two possible directions of development; for instance, ovarioles may be either gamic or parthenogenetic. The factor which determines which direction the structure will take in its development is the concentration of a substance, let us say, in the embryo at the time that determination of the structure takes place. If this concentration is high, development will be in one direction; if it is low, development will proceed in the other direction. After determination no change can take place in the direction of development.

Shull (1930b) describes one type of intermediate aphid which contains a gamic egg at the base of one ovariole with parthenogenetic eggs and embryos beyond. The presence of the gamic egg in the ovariole necessitates a gamic germarium at the end of the ovariole, while parthenogenetic eggs and embryos in the ovariole require that the ovariole be of the parthenogenetic type. Unless there are two germaria attached to one ovariole, one gamic and one parthenogenetic that produce eggs in that order it is necessary to conclude that the original germarium was gamic and that it was changed to the parthenogenetic type. Shull does not state which type of germarium was present when he observed the ovariole.

In the strain of aphids used in the study of differentia-

tion gamic eggs are produced by the germarium after the third instar only. If this is true also of the strain used by Shull at least one germarium of the above intermediate must have been altered several days after birth, and consequently after embryonic segregation. The above intermediate may be an unusual exception; nevertheless, it indicates that structures once determined might be changed in their nature and that such changes may occur after birth.

Other intermediates described by Shull (1930b) supplement the evidence that modification of structures after birth is possible. These intermediates are characterized by the following types of structures: (1) eggs which are partly gamic and partly parthenogenetic, (2) colleterial glands reduced in size, (3) hind tibiae partly swollen.

As all these intermediates were produced during a change from gamic to parthenogenetic females it is possible that the intermediate eggs were produced first as gamic eggs and later changed toward the parthenogenetic type. The only time such a change could occur is after the third instar, as gamic eggs are not produced until this time. There remains the possibility that the eggs were intermediate at the time they passed from the germarium into the ovariole. The germarium in this case would be probably intermediate also. This would exclude the necessity of a change after birth as parthenogenetic eggs are produced during the embryonic period and it is conceivable that intermediate eggs could be produced at the same time.

The intermediates with partly developed colleterial glands and hind tibiae offer somewhat weightier evidence that the combination of factors that cause intermediates is active during postnatal development. Both the colleterial glands and hind tibiae are differentiated after birth, hence whatever inhibited their full development in these intermediates must have been operative at the same time.

The recent analysis of intermediate-winged aphids by Shull (1937) indicates that in this type of intermediate the

order of determination is not the only cause of the production of intermediates.

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## SHORTER ARTICLES AND DISCUSSION

### FRESHWATER JELLYFISH RECORDS SINCE 1932

As so many isolated and scattered references to freshwater jellyfish have appeared in recent years, I have assembled for ready reference all American records, both published and unpublished, that have come to my attention since Dejdar's (1934) monographic treatise, "The Freshwater Medusa, *Craspedacusta sowerbii*." His list carries the known records through 1932. The last American entries are those of Kraatz (1933) for Ohio, and Brooks (1932) for Pennsylvania.

Dejdar appears to have inferred (p. 674) that the slow sand filters of the Washington, D. C., filtration plant from which I reported *Craspedacusta* in 1927 (1927) lay close to Great Falls, Md., from above which the city's water supply is taken. These particular filter beds, in truth, lie at a distance of 17.1 miles by underground aqueduct from Great Falls. There are two interruptions to the direct flow of the water, the first at the Dalecarlia Reservoir, 9.5 miles from Great Falls, and the second at the McMillan reservoir, 7.6 miles by aqueduct from Dalecarlia. The water is drawn directly from this second reservoir for filtering at the filtration plant located at Michigan Avenue and First Street, N.W. The Dalecarlia reservoir lies in a natural valley and receives some surface drainage from a well-protected area; the McMillan reservoir, however, is surrounded on all sides by ample concrete gutters and receives very little if any surface water.

No jellyfish have ever been observed in the Washington water supply system since the original and unique find of 1927. But Mr. J. W. Keys, who first discovered medusae in a "pot-hole" along the river shore just above Great Falls that year, secured additional specimens two years later, August 18, 1929, from the same pot-hole. These he presented to the National Museum. Miss Lillian Cash and Miss Edith R. Keleher, who each fished a number of specimens from Black Pond, Va., on August 11 and 27, 1929, respectively, also turned them over to the museum. This pond lies about one and a half miles below Great Falls and just below the mouth of Difficult Run. The pond is separated from the run by a rocky cliff. Lying about a hundred yards or so from a bend in the river, Black Pond at extreme high water is on occasion overflowed by the river.

In September of 1930 we determined a specimen of *Craspedacusta sowerbii* for Professor P. C. Bibbee, of the Concord State Normal School, at Athens, W. Va. This he had collected in West Virginia, presumably near Athens, in Mercer County, in the southern, mountainous part of the state. Athens lies at an elevation of 2,596 feet. It is a first record for the state.

About mid-August, 1931, Mr. Stanley G. Jewett, of the U. S. Biological Survey, stationed at Portland, Oregon, sent the National Museum seven specimens of *Craspedacusta sowerbii* (kindly determined for us by Dr. H. B. Bigelow) which he had obtained from the Willamette River, near Portland (Portland Harbor). In a recent letter he writes that the jellyfish seem never to have reappeared in the river. He took pains to inquire of friends who could be expected to have had knowledge of any recurrence of them. This is a first record of the occurrence of the freshwater jellyfish under natural conditions on the west coast.

On September 20, 1932, Mr. G. L. Hackleman, of Vandalia, Ill., sent in a sketch of a freshwater jellyfish, an undoubted *C. sowerbii*, which he had observed in numbers in a rock pool on the grounds of his Old Capitol Nursery Co. Van Cleve (1936), however, antedates this Illinois record by specimens found in a concrete garden pool in the same city by Mr. D. J. O'Donnel. The medusae were first noticed in 1931 and, although the pool was drained that winter, they were again seen on September 5, 1932, but disappeared by the 20th of the month. Van Cleve mentions also the discovery of jellyfish in a concrete fish and lily pond at Carmi, Ill., by Mr. John Cralley. These endured from August 8 to September 20, 1933, but failed to return in either of the next two summers, although a close watch was kept for them.

Allyn and Rettgar (1933) found large numbers of medusae of *Craspedacusta* in an old gravel pit three miles north of Terre Haute, Ind., first on August 20, 1932. The animals continued through August and some indeed persisted as late as October 10. This body of water is "totally shut off from the Wabash River, and received its water by drainage from the adjacent land. It is approximately 100 yards wide and one fourth of a mile long. So far as known, it has never been stocked with fish, but is used to a great extent by water birds during migration." Although no definite record was kept of the reappearance of these medusae in this gravel pit, Dr. Allyn writes me that they have been noted on three different occasions since 1932, that either they did not

appear every year or else escaped notice at one time or another. However, they were found in this gravel pit again in 1937.

In July, 1933, considerable numbers of jellyfish appeared in a goldfish pond in the garden of the Reverend William B. McIlwaine, of Alexandria, Va. The water supplying this pool comes from the local water mains. I have just learned from Mr. McIlwaine that the jellyfish appeared only the one year in his pool.

About this same time, Professor J. W. Bailey, of the University of Richmond, brought me several of these medusae from an old quarry pool near that city. In a recent letter he states that they were also found in 1935 and 1936 in three different quarry pools several miles apart, and again in 1937, but apparently not in 1934 unless they escaped notice.

Kraatz in 1933 reviewed the Ohio records that had been published at an earlier date by Baird (1932) and noticed by Dejdar. This same year, also, Woodhead (1933) found jellyfish in the Huron River near Ann Arbor, Mich.

The next year, 1934, Mrs. Inogene Robertson (1934) reported the appearance of freshwater jellyfish in a pond a few hundred feet from Lake Erie at Bay View, Lackawanna, a suburb of Buffalo, N. Y. From Texas Cheatum (1934) reported, July 25, 1934, literally thousands from a small artificial pond located near the city limits of Dallas. A number of specimens from this pond were collected by Dr. C. E. Burt that summer for the National Museum.

Two years later, October, 1936, Mr. Alfred C. Weed, of the Field Museum in Chicago, informed me that Mrs. William Pappas, of San Antonio, Texas, had been having a great deal of trouble with freshwater medusae in her garden pool that summer. They seemed to be present at almost all seasons, but became very numerous in warm weather. Mrs. Pappas told Mr. Weed that the jellyfish did a great deal of damage to the goldfish which were kept in the pool, that apparently whenever one touched the tail of a fish it seemed to cause such an injury that a portion of the fin dropped off. This is the first instance of which I have ever heard in which these jellyfish had caused injury. In compliance with a request made at that time for specimens, Mrs. Pappas forwarded several preserved in formalin to the museum the following summer, stating that the jellyfish had reappeared in her pool on July 27, 1937.

Harbaugh (1937) established a first record for Kansas when he reported the presence of a number of specimens in a private fish pool in the town of Manhattan during the month of July, 1936.

Iowa's first record was made the same year, when Mr. W. W. Aitken (1936), of the State Conservation Commission, found the species in a gravel pit called Avon Lake, ten miles southeast of Des Moines. They lasted here for a month, from August 20 to September 20, 1936. It was this same fall that Mr. Arthur W. Weidner, of Gettysburg College, Gettysburg, Pa., wrote the National Museum that he had found thousands of jellyfish in an old quarry close to town. This excavation had originally been made for clay, but was now filled with water. Mr. Weidner writes me that, although he watched the water in this quarry daily during the months of September, October and November, 1937, the jellyfish did not reappear.

On August 20, 1936, Atwood and Steyermark (1937) collected several medusae from a rock-margined pool along a "shut-in" of Marble Creek, near Fredricktown, Mo.

In a very recent summary, Breder (1937) lists several occurrences that have received little or no published attention: (1) "an outbreak of jellyfish in the water supply of Birmingham, Ala., during late July and early August of 1933," and again the following year; (2) Brady's Pond, Staten Island, where Mr. William T. Davis (1937) discovered freshwater jellyfish the first time in 1933 and where they have been seen again each year since that time, including 1937, a very consistent record, in view of the otherwise apparently sporadic and irregular occurrence of the animals; (3) Mr. Davis's (1937) report that jellyfish had also been found in Cranberry Lake, Sussex County, N. J., on August 29, 1937, by Miss Jane De Puy; (4) Franklin, Ind., where some fishermen found medusae in an old gravel pit near Sugar Creek, about seven miles east of Franklin, late in September, 1931. Dr. Naomi Mullendore, of Franklin College, who informed Breder of this discovery, added that no medusae were found the following year, or in 1936 or 1937, and no hydroids at any time.

On August 16, 1937, Dr. Dayton Stoner, of the New York State Museum, had his attention called to the occurrence of *Craspedacusta* in a fish pond at Loudonville, N. Y., three miles north of Albany. Notes upon the discovery and some observations upon the animals themselves have recently been published (1938).

On August 24, 1937, the National Museum received a number of

specimens from Dr. Kimber C. Kuster, of the Bloomsburg, Pa., State Teachers College. They were found in an abandoned limestone quarry filled with spring and surface water. This quarry is located about four miles east of Bloomsburg, near Almedia, Columbia County, and about one-half mile north of the Susquehanna River, at an elevation of 480 feet above sea level and perhaps 30 feet above the river. Medusae were collected in the quarry pool. They also developed in a laboratory aquarium containing water and water weed, *Elodea*, brought from the pool. Dr. Kuster has submitted a note regarding this find to *Science* for publication (1938).

In *Science* for November 26, 1937, Hamaker and Milne (1937), of Randolph Macon Women's College, report *Craspedacusta* from Crystal Lake, 14 miles south of Lynchburg, Va., while in the December 17th issue, Quick and Matthews (1937), state that freshwater jellyfish taken in Sandy Lake, Stoneboro County, Pa., by John Hines in 1936 were again found in the same body of water in 1937.

Under the date of January 25, 1938, I received a most interesting letter from Miss Eloise Kuntz, of the University of Washington, who tells me that last November she "found medusae in the sixteen tentacle stage in her twenty-gallon aquarium. They were common during the whole month of November, and were seen in small numbers during December." During that month two tropical fish were added. On January 20 a few medusae were still to be seen. None developed beyond the sixteen tentacle stage. She also found hydranths entangled in the algae and debris on the glass sides of the tank. As early as 1927, writes Miss Kuntz, Professor Kincaid observed large numbers of very small, immature medusae in a tank in the University Department of Fisheries, where they persisted for about two weeks. Dr. J. E. Lynch, of the Department of Fisheries, informed Miss Kuntz that hydranths were present in one of their tanks all during this past winter of 1937-38. So far, no outdoor record has been established for the State of Washington.

I have tried to give as complete a résumé as possible of the occurrence of *Craspedacusta sowerbii* in the United States from 1932 onward, so that it may be used in connection with Dejdar's masterly account. Thus, all known American records may be conveniently referred to.

I believe that the increasing number of records through the years is but a simple function of population increase, rather than



any actual increase in the number of occurrences. The greater the population, the greater the chance that these briefly enduring forms will be seen by some one.

In America, to date, *Craspedacusta sowerbii* has been noted in the District of Columbia, in eighteen<sup>1</sup> different states and in the Panama Canal Zone. The states are: Alabama, Georgia, Illinois, Indiana, Iowa, Kentucky, Michigan, Missouri, New Jersey, New York, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Virginia, Washington (only in aquaria) and West Virginia.

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<sup>1</sup> Since the above was set up, David Causey (1938, *Science*, 88: 13) has recorded two observations establishing the occurrence of *Craspedacusta* in Blue Lake near Prescott, Arkansas, late in the summer of 1927, and in 1937 in a pond near Stamps, in the same state, also late in summer. This increases the total number of states to nineteen.

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### WHITING'S HYPOTHESIS AND PTEROMALUS: A CRITIQUE OF DOZORCEVA'S (1936) STUDY<sup>1</sup>

DOZORCEVA (1936) investigated the inheritance of the "sex-linked mutant red-eye color" in *Pteromalus puparum*, in relation to the theory of sex-determination elaborated by P. W. Whiting for *Habrobracon*, and concluded that the theory accommodates her facts.

In view of the significance of work of this sort for an understanding of the mechanism of sex-determination in arrhenotously parthenogenetic forms a close examination of her work seems desirable.

Mutant red-eyed males (the red-eye color being recessive to the normal dark) were mated to normal females, and some of the F<sub>1</sub> females were backcrossed, while others were allowed to lay parthenogenetically. In the latter case males only were obtained and these consisted of red-eyed and dark-eyed forms in equal numbers. In the back-crossing experiments three categories of results were obtained: (1) "Either only normal individuals were produced" or (2) "normal males and an insignificant number of red-eyed females" or (3) "*vice versa*" (presumably meaning "red-eyed males and an insignificant number of normal females"). Dozorceva supplies a table of figures in addition to this general statement, but although it will be unnecessary to deal with it in detail here it can readily be shown to be inconsistent with the demands of Whiting's theory.

To facilitate discussion it is desirable to recount the postulates involved in Whiting's theory. They are: (1) The females are heterogametic (XY); (2) males of two sorts, (X) and (Y), re-

<sup>1</sup> Read at the Edinburgh Meeting of the Genetical Society, June, 1937.

sult from parthenogenetic reproduction; (3) fertilization is differential and selective in that an egg can be fertilized only by a sperm bearing a sex-chromosome of a kind contrasted to that borne by the egg itself; (4) only rarely does fertilization result in diploid homogamety, and the individuals so produced are males.

The results of the parthenogenetic laying by the  $F_1$  females is claimed by Dozorcheva to be in accord with Whiting's hypothesis, and while this is true it is no less true that the same result would be obtained whether the females were hetero- or homogametic if the factors for eye-color happened to be autosomal.

According to the theory we should expect two categories of results in the backcrosses according to the nature of the relationship existing between the factor for eye-color and the sex-chromosomes in the females. On the one hand, if the factor for normal eye-color is associated with a sex-chromosome of the same kind as that carried by the inseminating male there would be, in the absence of crossing-over: (1) Red-eyed females from fertilized eggs carrying the recessive factor; (2) red-eyed males from unfertilized eggs carrying the recessive factor; (3) normal-eyed males from eggs unfertilizable by the sperms concerned; and this class should be as numerous as (1) and (2) together.

Crossing-over would result in the addition of another class, that of normal females, and a shift in the numbers of normal and red-eyed males according to the degree of crossing-over.

On the other hand, if the factor for normal eye-color is associated with a sex-chromosome of the opposite kind to that carried by the inseminating male we should expect, in the absence of crossing-over: (1) Normal females from fertilized eggs bearing the normal factor; (2) normal males from unfertilized eggs carrying the factor for normal; (3) red-eyed males from unfertilizable eggs; and this class should be as numerous as (1) and (2) together.

Crossing-over would mean the addition of another class, that of red-eyed females, and corresponding alteration in the relative numbers of the kinds of males.

If we now compare these expected results with what she obtained and if we make every allowance for a 7.63 per cent crossing-over in eight of the ten backcrosses made and for 28.27 per cent in the others it will be seen that it is impossible to derive any of her three categories of results if the genetics of sexuality and

eye-color in *Pteromalus puparum* proceed according to the theory of P. W. Whiting.

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### THE FAILURE OF HOST GENOTYPE TO AFFECT CROSSING-OVER IN AN IMPLANTED OVARY IN *DROSOPHILA MELANOGASTER*

It has been shown by Schultz (Morgan, Bridges and Schultz 1932, 1933 and 1935) and confirmed by several other investigators that in *Drosophila melanogaster* and *D. pseudoobscura* (MacKnight 1937) the presence of an heterozygous inversion in one pair of chromosomes causes an increase in crossing-over in the other chromosome pairs. Schultz (1933) has attempted to explain this effect on a purely mechanical basis. Schultz's explanation, however, does not explain at least two known facts: (a) a long inversion has no effect on crossing-over in the chromosome pair which bears it. For example, the per cent. of double crossing-over obtained by Siderow, Sokolow and Trofimow (1936) for the yellow-forked interval from females heterozygous for the *sc*<sup>9</sup> inversion is 5.09, that obtained by Steinberg (1936) for the same interval from females whose chromosomes were normal is 4.6; (b) the increase in crossing over per unit map length caused by heterozygous inversions in other chromosomes is directly proportional to the metaphase length of the affected chromosome (Steinberg 1937). The present authors felt, therefore, that the explanation of this phenomenon had to be sought in another direction. In view of the marked physiological effects which most, if not all, inversions cause in the flies that bear them it was thought that possibly some physiological change was responsible for the interchromosomal effects of inversions on crossing over. The experiment reported below is an attempt to test an hypothesis of this sort.

Ephrussi and Beadle (1935) showed that transplanted ovaries may become attached to the host oviducts and that offspring may be recovered from the implanted ovaries. The same authors Beadle and Ephrussi 1937) showed that crossing-over may occur

in the implanted ovaries. Since it is known that the Curly and Payne inversions when combined cause a 285 per cent. increase in crossing-over between yellow and echinus (Steinberg 1936), it was decided to implant ovaries from mature female larvae heterozygous for yellow and echinus ( $\frac{y}{ec}$ ) into mature female larvae heterozygous for these two inversions

$$\left( \frac{Cy \ C_2L \cdot C_2R \ cy}{Pm}; \frac{C_3 \ LP \ Dfd \ C_3 \ RP \ ca}{H} \right).$$

When the host females eclosed they were mated to  $y \ ec$  males. Crossing-over was followed in the offspring which were known to have arisen from eggs derived from the implanted ovaries. Controls consisted of a backcross of  $\frac{y}{ec}$  females to  $y \ ec$  males. Table 1 shows a summary of the data.

TABLE 1

SUMMARY OF THE DATA OBTAINED FROM THE FOLLOWING 2 CROSSES: (a)  $\frac{y}{ec} \text{ } \varphi \varphi$

X  $y \ ec \text{ } \sigma \sigma$  (CONTROLS); (b)  $\frac{Cy \ C_2L \cdot C_2R \ cy}{Pm}; \frac{C_3 \ LP \ Dfd \ C_3 \ RP \ ca}{H} \text{ } \varphi \varphi$  WITH

IMPLANTED  $\frac{y}{ec}$  OVARIES X  $y \ ec \text{ } \sigma \sigma$  (TESTS)

	$\varphi \varphi$				$\sigma \sigma$					
	$y$	$ec$	$y \ ec$	++	$y$	$ec$	$y \ ec$	++	Per cent. cross-over	N
Controls	1,229	1,215	36	44	1,239	1,236	59	30	3.3	5,088
Tests ..	90	87	1	4	106	105	6	4	3.7	403

It is clear that no effect of the inversions present in the host was manifested upon the crossing-over shown by the implanted ovary. (The difference between the control and test crossover values is  $0.4 \pm 0.3$ ). Although the data indicate that crossing-over is a function of the genotype of the ovary in which it occurs (at least when ovaries of mature larvae are transplanted) it does not eliminate the possibility of such an effect as we have postulated; it merely indicates that if there is such an effect other methods than those employed here must be used.

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### THE GENUS AND SPECIES IN RELATION TO EVOLUTION AND TO SYSTEM

THE problem of a definitive and uniform relation between species and genus stirs into life all the age-old problems of analysis. The concept of nature inherited from earlier thought was eminently classificatory. The model of medieval science was a neat symmetrical hierarchy, something on the pattern of a dove-cote, with at the apex a single pigeon-hole, in the next lower row two pigeon-holes, below this four pigeon-holes, and so forth. This symmetrical scheme is an unconscious background or motivation of later thought. The dichotomous arrangement is not insisted upon; but classification is expected to approach it, and the division of one genus into a hundred species while another divides only into two is felt to be anomalous. Especially insistent is the expectation that the classes of things will arrange themselves into a series of definite ranks or levels as we proceed from individual specimens to a most inclusive class. In this case we ought to be able to define the species, the genus, the family and phylum, etc., in unambiguous terms. This conception of a symmetrical hierarchy is encouraged by experimental science, for example chemistry, which reaches a neat classification of its substances into elements, groups and compounds. There is a conspiracy to overlook the artifactual or unnatural origin of the substances and processes analyzed by experimental science, and the degree to which the formulae of experimental theory define uniformities of experimental procedure rather than uniformities of natural articulation.

It was always realized that the dove-cote classification of things breaks down when we pass from the species to the individual. No one expected, that is to say, an approximately equal number of occupants in the different species. The members of one species

might be countable, those of another species innumerable. But why, in this case, should we not expect one genus to possess only a few species and another a great many. There is a sense in which every species is an individual entity, covering just such an area of space, enduring through some particular interval of time, and portraying a unique history. A species is a natural unity, with interdependent parts and conserved in its existence and character by interbreeding, pervasive geographical climate and other conditions. What *natural* principle could set a maximum or a minimum to the number of species bearing the same generic character?

In truth, of course, the whole conception of a dove-coted or neatly pigeon-holed nature is to-day obsolete. It is a scholastic conception proper to the Middle Ages and invalidated by a dynamic science. In its place we have the concept of a nature in evolution, a nature which proceeds always in individual units, these units being subject to change. There can be no question of a system of nature, nor of a single, completed and definitive classification. We see that the old dichotomous classification dimly envisaged the truth that new forms appear as differentiations of earlier forms; but this is only to recognize, in one of its many aspects, the radical continuity of nature. Classification assumes, on the contrary, a radical discontinuity of nature, moderated by the appearance of an inexplicable likeness among the discontinuous parts. Evolutionary science, contrasted with merely systematic science, thus moves to radically opposite concepts of fact and modes of analysis. It sees in nature an articulate complex of spatio-temporal flow, analogous to a fibrous tissue the particular fibers of which may divide, cross and even re-integrate in unpredictable and innumerable diverse ways. In face of a world of this sort, classification becomes merely the first tentative step to a disentanglement or reconstruction of the whole fibrous tissue, in its historic growth; and while the preliminary classification implements this historical reconstruction, it is also subject to revision in the light of its findings.

The specific and generic discontinuities which appear in a non-historical and merely spatial survey give place to spatio-temporal continuities when we turn to an evolutionary survey. It is possible, within the short temporal span of observed fact, to follow the continuous articulation of nature. We see hybridization, the emergence of new form, the extinction of old form, etc. But our reconstruction of the larger past, *i.e.*, the larger movement of nature, is of course dependent upon hypothesis based on present

fact; and this means that a tentative analysis and classification of existent form is the preliminary to an evolutionary understanding of natural movement. But we must never make definitive this preliminary classification, since to do so would be to confine inquiry within arbitrary and casual dogma. This is just what we should be doing if we made definitive the distinction between the genus and the species.

It would seem that if there be a radical discontinuity in nature, proper to the establishment of a definitive classification, that discontinuity exists only at the microscopic level. Certain macroscopic characters may be determined by microscopic genes or other entities. In nature, however, these self-determinate entities, if they exist, exist as factors in natural units and not as natural units themselves. The individual plant, and even the individual cell, exist as organic complexes within which these factors work; and the individual units can not be defined merely in terms of the discontinuous elements. In actuality, accordingly, the discontinuous character of nature remains subordinate to the blended, interdynamic or continuous aspect of nature. Never in a natural science like botany, nor for that matter in a *natural* chemistry, shall we have the precise and definitive typification that we find in experimental chemistry.

There is no reason, however, why we should not prosecute, alongside the natural sciences of life, experimental disciplines analogous to experimental physics and chemistry. The classifications and definitions of such disciplines would hold of experimental and artifactual processes, and could not be directly attributed to natural fact. But they could—or rather we should say they already do—cast light on the processes of nature, and constitute a useful auxiliary to the natural as distinct from the experimental scientist.

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### SOMATIC CROSSING-OVER AND SOMATIC TRANSLOCATIONS

AN analysis of mosaic spots on diploid individuals of *Drosophila melanogaster* has led to the conclusion that they are the results of somatic exchanges between homologous chromosomes at homologous loci (Stern, 1936). Recent evidence obtained in the endosperm of *Zea mays* (Jones, 1938), on the other hand, has been thought to demonstrate the occurrence of somatic non-homologous exchanges (translocations) involving random groups of chromo-



somes. This interpretation is based on the finding of twin spots showing phenotypic changes related to genes located in non-homologous chromosomes.

On the basis of the translocation hypothesis, Jones suggests that the mechanism of mosaic production in *Drosophila* is also of the nature of random exchanges. Such an assumption seems to conflict with the fact that no spots resulting from somatic exchanges involving non-homologous chromosomes have been observed. Provided that the frequency of such exchanges is of the same order of magnitude as that of homologous exchanges, ample opportunity for their discovery had been available in experiments in which loci of both the first and third chromosomes had been suitably "marked" (Stern, *l.c.*). However, Jones suggests that in *Drosophila* cells resulting from non-homologous exchanges generally may be so unbalanced due to the possession of duplications and deficiencies that they will not reproduce and thus not give rise to observable spots. According to this view the mosaics actually obtained are regarded as being the rare surviving cases in which random exchanges happened to affect homologous loci.

Although it is true that many unbalanced conditions are not compatible with survival of cells, certain experiments show that at least some such constitutions result in observable mosaic conditions. They were obtained in flies in which the exchanges involved regions heterozygous for an inversion or a ring condition of the chromosome (Stern, *l.c.*, pp. 685-718). Here even homologous exchanges mostly lead to the production of nuclei with unbalanced chromosome sets. The resulting cells produce spots of characteristic and unique appearance. They are restricted to single setae of sub-normal length and diameter in contrast to the spots obtained in flies of regular chromosomal constitution which enclose one to many setae of normal size. The very rare occurrence, if not the complete absence, of abnormal spots in flies of regular constitution is a strong indication that somatic exchanges in *Drosophila* occur prevailingly, if not exclusively, at homologous loci. The term somatic crossing-over for such exchanges seems to be an appropriate one.

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## THE AMERICAN SOCIETY OF NATURALISTS THE LIFE HISTORY AND PERSONALITY OF THE CHIMPANZEE<sup>1</sup>

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"IN the title 'The mind of a gorilla' you assume what should be proved." This was the critical comment of my colleague, Raymond Dodge, when I proposed so to label an account of the behavior of a mountain gorilla. I replied that "mind" was the best term I could discover for the facts which I had gathered. So likewise in this address I am assuming that personality is the correct and adequate term for what is now known concerning the integrated behavior of the chimpanzee. Indeed, in my present thinking there is no question about the reality of chimpanzee mind, individuality, personality. If you dislike psychological terms, it is your privilege to substitute whatever symbol seems more fitting. I ask only that you acquaint yourself with the essential features of anthropoid behavior before pronouncing my assumption unpragmatic or illogical.

It is my purpose in this address to picture as serviceably as I may in the few minutes allotted me the psychological characteristics of the chimpanzee, to suggest grounds of the animal's peculiar scientific usefulness, and to invite attention to some methodological desiderata which we naturalists are prone to ignore or slight. Speaking primarily from first-hand acquaintance with the

<sup>1</sup> Address of the president of the American Society of Naturalists, Richmond, Virginia, December 30, 1938.

facts, I shall do my best to render a somewhat heterogeneous array of observations meaningful and entertaining. The cinema record from the Yale Laboratories of Primate Biology which will be shown at the end is intended as antidote for boredom.

To put methodological considerations first requires no apology. I should like then to point the contrast between naturalistic and experimental inquiry, since to-day we are assembled as members of an honorable scientific organization whose name directs our thoughts backward and reminds us that many scientists think of the naturalist as having been replaced by the experimentalist. I beg to offer contrary opinion and to maintain that the interests, objectives and methods of the two are supplementary, and neither substitutes nor alternates. For the naturalist, with minimal disturbance of organism or environment, attempts to find out about life as it is lived; while the experimentalist, with some definite problem in mind, seeks so to control the conditions of observation that solution shall be facilitated.

True, the development and application of experimental techniques and skills mark a path of progress toward more exact knowledge and better understanding of animal life and relations. Witness the results of experimental versus naturalistic studies of color vision. But, nevertheless, naturalist and experimentalist may be equally worthy of support, admiration and emulation. Each, it may be argued, is necessary to safely efficient biological advance, since we need to know animals intimately, sympathetically and disinterestedly as natural objects if we are to use them with maximal effectiveness in well-planned experiments. It would be ideal, as I see the situation, if in each of us biologists might be combined the interests and abilities characteristic of the best in field observer and laboratory worker.

Contrasted with rapid progress in the control and measurement of the environmental factors affecting behavior is the relative neglect of the nature and immediate

condition of the animal observed. Continuing in the tradition of the naturalist, the experimentalist too frequently accepts his animal subjects as given in nature and proceeds to use them without adequate knowledge of prior history and immediate condition. Surely it should be unnecessary in this family group to argue for the importance of suitable biological subjects and definite knowledge of their essential characteristics. For it is obvious that in many psychobiological, sociological or more conventionally physiological studies, it is absolutely essential to reliability and interpretability of observations that varied aspects of the life history of the organism—developmental, nutritional, reproductive, disease, experiential—be known. In such case our subjects should be bred in a laboratory colony, reared and maintained under appropriate conditions, and used as relatively standardized observational materials. How amazing, when one pauses to consider it, the difference between the requirements of a chemist relative to the purity, describability and dependability of his materials and those of the physiologist, psychologist or sociologist. In case of self-pity we may claim that ours in varied respects is a more difficult task than is that of the physical scientist, but against this must be placed the admission that one satisfactory organism is likely to be of more value than scores whose special suitability is merely taken for granted. Actually the two are incomparables, as are correct and incorrect observations. It is my earnest plea, therefore, that we pay more heed to the origin, nature and status of our animal subjects and to their immediate fitness for experimental use.

Freed from varied superstitions concerning living things, it is now our opportunity and obligation to strive to shape them in accordance with experimental requirements and on occasion to specification. What a far cry biologically from the naturally given animal to that which is experimentally produced and conditioned as biological test or experimental object. The organism is continuously in flux; varieties and variabilities are omnipresent

and of the utmost importance. Upon them, changes both progressive and retrogressive depend.

It is evident that my mind has turned to the problem of species. I wonder how many of us in this learned company could readily and with certainty identify the three genera of great apes—gorilla, chimpanzee and orang-outan—in all their stages of life history. It is not my present intention to try to inform you about the content and differentia of our own order, the primates. Instead I would inquire humbly, for I am not a taxonomist, whether the terms genus, species and race have the same significance when applied to man or chimpanzee, or if not, why not? We hear commonly about genus *Homo* and genus *Pan*; about races of man and species of chimpanzee. But if species of the latter why not also of man, since human races appear to differ more from one another than do the so-called species of chimpanzee? Have we perchance, in formulating this seemingly naïve question, stumbled upon a persisting biological myth; namely, that man as lord of creation stands unique and incomparable, separated by a vast gulf from the remainder of the organic world? It seems that even in taxonomy we are reluctant to apply to ourselves the rules which we habitually apply to our nearest living kin, the anthropoid apes. But, despite our prejudices and superstitions, truth marches on and wisdom and enlightenment increase with the extension of knowledge. In the Yale Laboratories of Primate Biology, where by the broad-minded liberality of Yale University and the Rockefeller Foundation it is my privilege to work, we are seeking by several routes to cross the gulf between man and anthropoid ape, that we may know ourselves and them in true genetic relation and perspective. By most of the paths progress is slow and halting, but in some directions the fruits of our labors are most encouraging.

My psychobiological knowledge of the primates stirs me to ask also whether the taxonomist may reasonably hope to proceed safely in his revision of the Order Pri-

mates without knowledge of the age, developmental status, normality and degree of typicalness of his specimens. Such taxonomically favorite characters as those of teeth, skeleton, skin, coat, body form, are known to vary with age, nutrition, state of health and other factors. Often this seems to have been ignored by species-makers, and as a result we have descriptions of a score or so of chimpanzee species. Perhaps we are too lenient with ourselves in this field, as well as in our studies of behavior. Certainly times without number specimens have been accepted as they chanced to come to hand, although clearly enough essential information about them was lacking. The argument which I have in mind runs thus: We can not hope to classify animals serviceably without knowledge of age and of those changes which characterize the life cycle. Even taxonomy is bound to natural history.

It is incomparably more illuminating to follow the life history of an animal from birth, or earlier, to maturity than to know it only in cross-section at some particular stage of development. Few biologists unfortunately have had opportunity to follow a chimpanzee through its decade of development. Such rare good fortune, as it has come to me in the past twenty years, together with the invaluable contributions of my associates in primate research, enable me to describe this ape in terms of three series of events: the sexual cycle, the reproductive cycle and the life cycle. Of necessity my account will be synoptic and far from ideally complete, for as yet we are ignorant of much that is important.

The chimpanzee sexual cycle typically occupies five weeks. Menstruation, genital swelling and oestrus are readily observable. Shortly after the completion of menstrual bleeding the genital-anal tissues begin to exhibit tumescence. Within one to three days the area has swollen to surprising proportions. Maximal swelling continues about ten days. In extreme cases the volume has been estimated as four liters. During the period of

genital swelling, and ordinarily only then, the female is sexually acceptable to the male. Her receptivity, and presumably also her sexual desire, appear to wax until a maximum is achieved during the last few days of genital swelling. It is during this interval, according to our observations (Elder, 1938), that ovulation and fertilization occur. Indeed, we have no evidence of conception in any other phase of the sexual cycle than during the last few days of maximal swelling. The human cycle differs notably from that of the chimpanzee in being about one week shorter, in the relative inconspicuousness or absence of genital swelling and oestrus, and in the reported occurrence of conception during other cycle phases than the period to which it appears to be restricted in the chimpanzee.<sup>2</sup>

By the cooperative functioning of male and female during the appropriate phase of an ovulatory sexual cycle, the latter may be converted into a reproductive cycle. Mating behavior may be initiated by either sex. Female receptivity is indicated to the male by the genital appearance of the female and also by her behavior. Either sex may be dominant. As yet we are ignorant of the life span of the ovum after the rupture of the follicle, and also of that of the spermatozoon in the reproductive tract of the female. Until these facts are reliably established, neither the time of ovulation nor that of conception can be stated with accuracy and certainty. With the possible exception of the Burr electrical variation method (Burr, Lane and Nims, 1936), no conveniently applicable procedure for the observation of ovulation is available.

Ovum implantation, as observed by Carl H. Hartman (Elder, Hartman and Heuser, 1938), is deep in chimpanzee as in man, instead of superficial as in the monkeys which have been studied. This would seem a very significant point of similarity between man and chimpanzee. Probably it will presently be discovered to be common to the anthropoid apes and man.

<sup>2</sup> Knowledge of human ovulation is still incomplete and observations are difficult to evaluate.

Gestational process from conception to birth requires about thirty-four weeks in chimpanzee, as compared with thirty-eight weeks in man, or approximately eight versus nine months. To judge from our observations, parturition as a rule is neither prolonged nor so difficult as to endanger the condition of mother or infant. Vertex presentation appears to be typical, and so also is placentophagia, either partial or complete.

Normally the infant is accepted by its mother as soon as it emerges from her body, carried about continuously, cleaned with lips and fingers, and finally permitted to cling to her abdomen, groins or breast, except as she handles it for purposes of care or of her own comfort.

Continuing this account of the reproductive cycle into the individual life cycle, we note that after as before birth the rate of physical development in chimpanzee is appreciably more rapid than in man. If body weight be taken as index of growth and development, it is discovered that the curve of growth for the ape is very like that for man, "being characterized," as we have recently been able to report from our anthropometric data, "first by an infantile period (birth to 24 to 30 months) during which the time rate of growth (velocity) gradually decreases; second, by a juvenile period (3 to 7 or 8 years) in which the time rate increases at a constant rate; and third, by a mature period (7 or 8 years to 11 or 12 years) during which the rate of growth gradually decreases to zero. The pattern of the curve of growth of chimpanzee is much more similar to that of man in quantitative and qualitative characteristics than to that of other mammals which have been studied. The major inflection point at the junction of the positively accelerated juvenile period and the negatively accelerated mature period coincides in chimpanzee as in man and other mammals with the physiological stage of puberty. The relative rate of body growth in chimpanzee during the positively accelerated pre-pubertal phase is about 25 per cent. per year as compared with 10 per cent. for man and 1000 per cent. for other mammals



in corresponding periods." (Spence and Yerkes, 1937, pp. 244-245.)

Chimpanzee infancy, as period of relative helplessness and utter dependence upon mother or other older individual, is a brief two years. Whether if left to its own resources in a highly favorable material environment the year-old baby would survive is doubtful. But certainly if it be two or more years advanced, it should be able to do so without special difficulty. Ability to walk, climb, exercise and play about may be achieved by the middle of the first year, and these develop apace during the latter half.

As in us, childhood, with its freedom from cares and responsibilities, is a period of extreme playfulness. It is the period of development which most strongly appeals to men who use these animals as subjects, whereas by contrast, and naturally, the period of infancy is more appealing to women. A more high-spirited, good-natured, responsive, affectionate and entertaining pet than the chimpanzee child is difficult to discover.

But with the onset of adolescence the stream of life tends to run less smoothly, for problems involving the adjustment of the physiological self to environment abound. There are conflicts of interest and desire; evenness of temper and playfulness become less predictable, and at times impatience, irritability or downright meanness may be manifest. To the friendly observer who has been on terms of intimacy with the individual from its birth, this change may be disturbing and question-provoking. It becomes increasingly difficult to get along with the ape, and especially so in the case of the female. As the biologist appraises the situation, the individual is on the threshold of maturity and racial functions have thrown their shadows before.

Sexual maturation requires from seven to ten years. It is attained somewhat more rapidly than by man in the tropics. The duration of the reproductive life of the female ape has not been measured. In our Yale chimpanzee colony there is one female who is still breeding at

an estimated age of twenty-six years. She seems old to us, but not senile. One would surmise that the reproductive period may not ordinarily exceed twenty years in duration. As for the length of life of the chimpanzee, our ignorance is complete. It is indeed a long task to rear a sufficient number of subjects and to follow their histories to death in old age from natural causes. I do not venture a guess as to the average span of life in nature or under favorable conditions of captivity.

It is not without significance for the interpretation of our results that until recently most of the behavioral studies of the chimpanzee have been made on young specimens. Chimpanzees they are to be sure, but how different psychobiologically from their adult selves.

The sexually mature or middle-aged ape, even if still friendly, gentle and cooperative with the observer, is no longer forthright and eager in play, overflowing with the joy of activity, even-tempered and fairly dependable. Instead he or she tends to be grave of mien, quiet, relatively inactive, attentive to the necessities of life, but somewhat impatient of everything else. Serious-mindedness is the most apt phrase. One may not with impunity joke carelessly or thoughtlessly with a really serious-minded acquaintance, whether man or chimpanzee! Beyond the first decade in its life history, chimpanzee playfulness and fooling behavior rapidly diminish until they become very rare. It is not that the individual has become unfriendly or morose; one has instead a sense of the gravity or weightiness of the duties and obligations of being an adult ape, for evidently in growing up they, like ourselves, gradually lose buoyancy and the tendency to be lavish of energy in activity and emotional expression. Aging it is, and by twenty years this primate ordinarily is as old behaviorally, and perhaps we can safely say psychobiologically, as we are at forty to fifty years. How fascinating and bewildering are the changes in attitude and activity from birth to maturity. It is to be hoped that some time we shall be able to record cinematographically

this varied series of anthropoid events, condensing a decade of development into an hour of observation.

At the moment I am unable to complete this thumb-nail sketch of chimpanzee life history by including the characterization of senility, since it has not been our good fortune to observe any individual in this phase of life. The oldest of my acquaintances are in their third decade, with no definite indication that they may not live for many years.

As Köhler has aptly said, "It is hardly an exaggeration to say that a chimpanzee kept in solitude is not a real chimpanzee at all" (1925, p. 293). It has become perfectly clear that only in a favorable social environment does the personality of this ape emerge and develop normally. It is for this reason that I now turn to the facts of social behavior and relations for material to complete this picture of chimpanzee life.

Behavioral development in the ape is from initial social dependence to dominance; from clinging or clasping to venturesome climbing and independent bread-winning. In the early months of postnatal existence the infant securely grasps the mother and is carried everywhere with her. Maternal care preserves and safeguards it, and both early and late maternal example and tuition facilitate habit-acquisition and adaptation to many types of problem situation. To a degree which it would be difficult to overestimate, the female of the species shapes the development of the individual. Indeed, it is not until childhood that the little chimpanzee escapes the surveillance of its mother, dares to face the unexpected and startling events of its world and to live abundantly in the company of its juvenile peers. Thenceforth and increasingly, child learns from child, and social contacts become more varied and exciting, if not more exacting as well. The playful personality speedily emerges, together with those social behavior patterns which later will determine the place and relations of the individual in the social order.

The behavior which the term dominance usefully desig-

nates is manifest during childhood, takes more definite form in adolescence, and hardens into habitual forms of expression in maturity. Ultimately, by its dominance-subordination attitude and behavior, the individual establishes its place in the social hierarchy. It may command the right to have its own way with its fellows in any situation, to impose its will and desire on the group no matter how unfair or inimical they may be to immediate social welfare; or, on the contrary, it may meekly accept a subordinate place and in a sense become enslaved. For in its extreme expression, dominance spells selfishness, injustice, tyranny, cruelty. The contrast between dominant and subordinate chimpanzee personalities is rich in significance for students of social science.

By virtue of hereditary constitution, as modified by individual experience, there appear two types of ape personality: the one constituted to command, and the other content to obey. To the former fall inevitably opportunities and demands for leadership, initiative, adventure, progressiveness and varieties of social service; and to the latter, the opportunity or necessity to follow, serve, consolidate advances. In our genus we think of the male as typifying dominance, and the female, subordination. Yet we know, from available facts, that the situation is by no means so simple and the dichotomy far from true. It is even clearer for the genus *Pan* that an individual, irrespective of sex, may be dominant and domineering or the opposite. In general, might makes for right in chimpanzee life, but physical prowess may be outmatched by intelligence, courage and self-confidence.

Physique, intelligence, temperament and experience, as objectively evaluated and describable in behavioral terms, stand forth as the essential determining conditions of those social behavior patterns which we have come to designate as dominance and subordination. Highly impressive and socially significant in ape as in man are such biological contrasts as robust and puny, bright and dull, well-adapted and inept, courageous and cowardly.

Even a hasty, incomplete sketch of the psychobiological characteristics of the chimpanzee must include such likenesses and unlikenesses to man as the following. The modes of sense, so far as known, are the same in ape and man, and so also the order of keenness and range in each. Perceptual processes, although relatively little known, evidently differ markedly in the two primate types in correspondence with differences in their manner of life as well as their physique. Undoubtedly there offers engaging opportunity for discovery in this psychobiological sphere, for without knowledge of its perceptual capacity it is difficult to imagine or predict the behavioral adjustments of an organism. As to memory, the chimpanzee is efficiently equipped, for it remembers well and long anything which vividly impresses it. Analyses with respect to memory content, detail and accuracy await future investigators. By contrast with memory as reproduction or revival of psycho-neurological processes, imagination, although frequently evidenced by the behavior of the ape, is of relatively low order, simple and incomparably less important than in man. Were this type of creative process more common, the animals might be expected to develop a spoken language. As a fact, they depend for intercommunication primarily on bodily posture, gestures and sounds; and of language as a system of symbols used serviceably for the expression of experience, they have none. It appears that man as thinking and talking animal is virtually unique. There are evidences in the life of the chimpanzee of what in ourselves we term ideational processes, insight and reasoning, but they are relatively infrequent, and at a stage of development and functional usefulness which suggests human infancy or early childhood. Perhaps more than in any other sphere, this ape resembles man psychobiologically with respect to its modes of affective expression. That it feels as we do may not be asserted with assurance, but it is clear that under conditions which affect us emotionally it manifests similar expressions.

A few additional glimpses of the chimpanzee personality as it functions in social situations should appear as high lights in the picture.

With a facility which from late infancy to maturity steadily lessens, these apes acquire attachments to one another and to human acquaintances. Particularly in face of such imperative needs of social service as in illness, injury, threat to safety or inability to meet a baffling situation, positive affective bonds give expression to gratitude, sympathy and fondness. One is not often left long in doubt about the attitude of the animal, for attractions or aversions tend to make themselves felt almost immediately: the former in pleasant or even flattering ways, the latter by actions which may be repelling or belittling. Along with many weak and transient attachments there are some which endure for months, years or perhaps even throughout life. It is no over-statement to assert that chimpanzee friendship or enmity for fellow ape or man may be exceedingly strong and permanent. Teasing, unfair or unkind treatment may almost instantly establish hostility and antagonism, whereas the opposite in treatment may induce appreciative friendliness. The significance of these facts for the social life of chimpanzees and the guidance of persons who care for or use them is too evident for comment.

It is a primary task of caretaker or experimenter to win the confidence and trust of his subjects. Affective attachment implies a measure of liking and of dependence, but not necessarily confidence. The latter is achieved, if at all, through patient, tactful and understanding treatment of the chimpanzee subject. To attempt to command or to hasten its acquisition is likely to be fatal to success. Individual differences in the granting and also in the depth of confidence are extreme. Some individuals grant it readily and completely, others not at all. Likewise some persons with facility command the trust of their ape subjects, while others have extreme difficulty in doing so. Ordinarily trust, whether among apes or between ape and man,

becomes mutual and establishes or reenforces attachment. This is the secure basis of success, facility and safety in handling the animals, whether as pets, stage performers or experimental subjects.

Confidence is also the essential basis of cooperation. Once an investigator has won the complete trust of his subject and its affection, he may count on obedience and cooperation to extraordinary lengths. To this end it is necessary only that he make the animal understand what he wishes, control and direct its interest, and free it from inhibiting fears. Under these conditions even irksome, distasteful or painful experiments may be conducted with the wholly voluntary cooperation of the trained and experienced chimpanzee. The concluding phrase is important, for unless previous experience has acquainted the animal with what is required of it in the experiment and with what may be expected to happen, it is likely to be timid and apprehensive, despite its friendliness toward the experimenter and its confidence in him.

Whereas formerly most of our exacting behavioral studies in the Yale Laboratories of Primate Biology were conducted under conditions of subject-confinement, restraint or coercion, more intimate acquaintance with the characteristics of our subjects and extended experience have taught us that in many instances voluntary cooperation of the free subject may be relied upon. This is an important discovery in methodology, for in not a few of our investigations coercion or force is unfavorable to the reliability and interpretability of results, and in all cases cooperation without restraint makes for safety, convenience and economy of experimental effort. In a word, then, attachment, mutual confidence, familiarity with the situation, freedom from intruding fears and willingness to cooperate on request or command, pave the road to success in most varied and exacting experimental quests.

Intimations of altruism may be discovered in the behavior of many mammals, but the evidences are most varied and abundant in the life of anthropoid apes and

man. Functionally viewed, an act of fellow service may be more or less unselfish, but it is doubtful that any act, even in man, is purely altruistic. It happens that "grooming" is a pattern of chimpanzee behavior which at times supplies convincing examples of social service. A simple form of this activity is familiar to all visitors to the zoo as flea-picking. For many types of monkey in captivity this is a favorite pastime. It may be mutual or self-directed, but in either case it is usually a toilet-making process instead of a search for ectoparasites. The chimpanzee grooms self or companion with skill and evident enjoyment. Lips, teeth and hands may be employed, and as the activity proceeds sounds frequently are made with tongue, teeth and lips. The behavior is not necessarily correlated with sex. It is rare during childhood, more frequent in adolescence and characteristic of the sexually mature individual, who may groom another individual irrespective of age. Often grooming is solicited, begged for, insisted upon or commanded, in accordance with the dominance-subordination relation of the individuals. This frequently appearing behavior pattern serves to keep the skin and coat clean and neat, to remove thorns, splinters, burs, chiggers, to cleanse or drain surface wounds or abscesses. Obviously such service is especially important for regions of the body inaccessible to the individual. Grooming often is necessarily a vicarious service, and whether willingly and eagerly or reluctantly performed by the groomer it constitutes social service and in so far may be considered altruistic. Chimpanzee fellow-service is not limited to grooming. I have selected this behavior from among many varieties merely for illustrative use.

If the impression has been given that the personality of chimpanzee is relatively uniform and constant, violence has been done to the facts, for there are individual patterns and types. One may as readily identify a familiar ape among many by its personality as mirrored in behavior as by its physical appearance. The student comes soon to recognize his subjects as in varying degrees pa-



tient, tractable, docile, suggestible, gentle, friendly, trustworthy or the opposite. There are preferred and neglected individuals in any group of experimental chimpanzees, for one may quickly grasp the requirements of a situation, cooperate perfectly and facilitate the work of the investigator, whereas another may be slow, unpredictable, relatively uncooperative or even refractory. There are good and ill-natured individuals, stable and unstable, calm and excitable, industrious and lazy.

Because amidst important differences the chimpanzee is incomparably similar to man in structure, development, physiological processes, behavior, social relations, susceptibility to disease, response to various educative or therapeutic measures, it should prove invaluable to science. Naturalistically viewed, it is a fascinating object, knowledge of which, although growing slowly for decades, is still fragmentary and sadly inadequate to the needs of investigators. Experimentally considered it stands as unique in potential availability, controllability and usefulness for attack on many problems of psychobiology, sociology and experimental medicine, to mention only a few areas of interest. The exploitation of this extraordinary resource for biological progress lags because of lack of interest, faith and material resources.

It is not by oversight that I have neglected to use observations and contented myself with description in general terms, for I count upon the cinema record which you are about to see to lend reality to my subject. Moreover, I have avoided what might seem like behavioral incident or anecdote.

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# THE EXPERIMENTAL ANIMAL FROM THE NATURALIST'S POINT OF VIEW<sup>1</sup>

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AMERICAN MUSEUM OF NATURAL HISTORY

THE naturalist is popularly supposed to be one who studies animals or plants under natural conditions. To-day there is an increasing group of naturalists who are concerning themselves with the productivity of natural populations and its bearing on forest, fish or game management. These workers have shown that wild populations are in a continuous state of change, that the balance of nature is usually unbalanced. In addition to the changing predator-prey relation and the cover-carrying capacity correlations (Errington, 1937) there are less tangible social integrations to consider. Langlois (1934) has showed that the black bass, *Micropterus dolomieu*, for example, can be reared more successfully in clear ponds free of vegetation and provided with artificial foods than under conditions which appear to be much more natural. "The occurrence of superiority and awareness of it, and of inferiority and awareness of it, in the case of bass leads to cannibalism," Langlois states, and to grow large bass crops one should adopt measures to make the fish more socially minded.

The principles of social integration in fish should be of interest to others besides fish culturists. Endocrinologists have described numerous sexual differences among fish, some under nervous control but others regulated by sex or pituitary hormones. The functional significance of these differences can be determined only by reference to the social life of the fish. Again, recent discoveries in paleontology have tended to bridge the gap between the classes of vertebrates. We can now trace with consider-

<sup>1</sup> Read at a Symposium of the American Society of Naturalists in joint session with the American Society of Zoologists, the Botanical Society of America and Section H, The American Association for the Advancement of Science, Richmond, Virginia, December 30, 1938.

able assurance the changes in the skull from fish to man. Although man is equipped with certain special endowments not found in other vertebrates, he is nevertheless a social animal and we may well ask where in this series did his social behavior first arise. If it should be possible to trace the components of his social behavior to their beginning in lower vertebrates, the next question is, What are the mechanisms responsible for this behavior?

A school of fish is held together by innate attractions. This is readily shown by rearing aquarium fishes (such as *Hemichromis bimaculatus*, etc.) from a very early age in isolation.<sup>2</sup> When placed in a tank with several species they will eventually school with their own kind (Noble and Curtis, 1939). Fish reared singly with other species also school with their own kind when given the opportunity. The size of the species concerned, the manner of swimming, the reaction of the resident fish modify the result, but basically there is a species attraction, as Picard (1933) assumed. Blinded aquarium fish school very feebly, if at all, and hence vision would seem to be the chief sense modality mediating schooling. Nevertheless, fish may not be attracted by an inborn image of their own species. Fish tend to move towards other moving objects and to react under different conditions in ways peculiar to their own species. The guppy, *Lebistes reticulatus*, for example, when seriously disturbed, moves against gravity and hence the group tends to aggregate near the surface of the water. Young catfish, *Ameiurus melas*, although attracted by motion, are also stimulated by contacts with their own kind, but whether this is a conditioned response has not been determined (Bowen, 1931). In some cichlids there seems to be an inborn attraction to particular motions. *Hemichromis bimaculatus*, isolated at only 5 days of age and reared for 77 days with *Cichlasoma cutteri*, returned at once to their own species when given the opportunity (Noble and Curtis, 1939).

Directly opposed to this cohesive force of the group

<sup>2</sup> This investigation was supported in part by a grant from the National Research Council, Committee for Research in Problems of Sex, and in part by a fund from the Josiah Macy, Jr. Foundation.

attraction, there is in most aquarium fishes, and presumably in teleosts in general, a continuous aversion for one another which expresses itself in a dominance drive. Every fish by threat or blow seeks to gain dominance over every other member of the school. There results early in life in such fish as *Xiphophorus helleri* a straight-line "pecking order," similar to that which has been described for the domestic fowl (Schjelderup-Ebbe, 1924). Fish near the top of the order have better access to food and mates (Noble and Borne, 1938). During periods of starvation, fish at the top of the order lose less weight. Large or heavy fish tend to dominate the others, but there are many factors which may give the advantage to a lighter fish. Mere familiarity with an area gives the resident a decided advantage over a newcomer. The former usually attacks first and, if there is not too great a discrepancy in weight, it will become dominant. Brighter or more energetic fish usually dominate. Merely cutting the sword from the tail of a male *X. helleri* usually drops him in the dominance hierarchy, but after a time he may regain his position by fighting. The order in a small school may remain constant for two or three weeks. Female *X. helleri* following parturition usually rise in the order. Dominant fish do not permit subordinates to fight near them without seeking to "punish" them. Apparently the gestures of the aggressive subordinate stimulate the dominant fish to exert its authority. Usually the fish second in order receives more blows from the despot than the one third in order, and the latter more than the one fourth in order, etc., but under certain conditions a fish low in the order may evoke the attacks of the despot far more than do the fish higher in the social order. Every fish in the school seems to know every other as an individual, and every subordinate appears to be awaiting an opportunity to revolt against the despot. An attack upon a despot frequently leads to a complete revolution within the group, each individual taking up a new position in the order.

At sexual maturity the dominance hierarchies are dis-

rupted by the sex drive of the males. Female *Xiphophorus* may still retain their straight-line order, but at that time even relatively small males will attack them. There results a new order with the females confining their blows to females, and the males to their own sex. This "chivalry" is not due merely to the greater strength of the males, for castrated males usually resume their attacks. Mature males would seem to have a latent sex interest, which becomes manifest in overt behavior only at intervals, and this keeps the dominance drive against females in check. The secondary sexual characters of the male serve to identify his sex, for if a small female has a sword artificially attached to her tail she is treated like a male by the other fish. Conversely, a male with his sword and gonopodium removed is treated like a female (Noble, 1938).

In nest-building fish, such as *Hemichromis bimaculatus*, the young when free of their parents establish straight-line dominance hierarchies regardless of sex (Noble and Borne, unpublished). At sexual maturity these hierarchies are modified in a manner different from that in the live-bearer, *Xiphophorus*. The breeding fish, usually the male, claims a territory containing a suitable spawning area and drives off other fish from this region. A subordinate male with territory becomes dominant over his despot and over other males which enter this area. Gravid females, attracted by the male's display and symbolic nesting movements, enter his territory. If two males are available, the female is attracted by the more conspicuous fish (Noble and Curtis, 1939). In other words, there is a true sexual selection in the sense of female choice. The courtship movements of the female *H. bimaculatus* are also stimulating to the male and her threatening gestures ward off predators from her eggs and young. It is therefore not surprising that the female as well as the male becomes conspicuous by an expansion of erythrophores during the breeding season.

Since all birds are egg-layers, the territorial habit is

well developed in this group. The territories, however, may be very extensive and have the secondary function of assuring an available food supply for the young. In many primitive birds, such as the night heron, the territories retain their piscine function of being primarily a place where sexual bonds may be formed. These are necessary in both fish and bird if the mates are to be held together as a pair during the breeding season (Noble, Wurm and Schmidt, 1938).

Fish which school more than *Xiphophorus* or *Hemichromis* are unable to maintain the straight-line hierarchies of these forms. Instead, as in *Barbus partipentazona* and *Danio malabaricus*, each despot may receive some blows in return from its subordinates. There results a peck-dominance system similar to that Masure and Allee (1934) described for the pigeon. This apparent confusion of hierarchies seems to result from the more compact groupings of these better schooling fishes and better flocking birds. At least in forms where the species attraction seems to be poorly developed, the dominance hierarchies tend to be better established. An exception occurs in the case of *Betta splendens*; the females develop straight-line systems, but the males are so aggressive that no true order is established. Dominance behavior has apparently a genetic basis. At least the white strain reared in our laboratory in the same tanks as colored varieties proved inferior to them even when greatly exceeding them in weight.

Dominance hierarchies, although well developed and of several types in fish, never reach the complexity found in higher forms. Weight becomes in these higher vertebrates of less importance than other factors in helping an individual secure dominancy. Many birds and mammals recognize not only the individual but the group. Resident hens when confronted by a group of newcomers distinguish the new hens from their own group (Skard, 1937). Jackdaws are also able to keep the group in mind, acting as a group against intruders of their own species

(Lorenz, 1931). No fishes apparently react as a group towards newcomers. In *Xiphophorus*, either a single newcomer, if sufficiently large, or a group of new fish will produce a revolution in the resident hierarchy. In the latter case each fish fights for itself against residents and introduced individuals. While the dominant resident usually remains the despot, a fish from either group may rise to second place in the order.

Although mammals, like fish, exhibit a variety of dominance hierarchies, a marked advance is found among certain forms especially the anthropoids (Yerkes, 1934). In the chimpanzee, subordinates are attracted to dominant individuals and frequently imitate them. Wheeler (1930) assumed that the dominance drive was a cohesive force in vertebrate sociology. At the fish and bird level it is exactly the opposite. Murchison (1935) described the hen as being attracted by the more dominant rooster. This, however, seems to have been a sexual attraction similar to that shown by the territory-seeking *Hemichromis* female. Dominance behavior in fish and birds begins long before sexual maturity and tends to keep individuals at a distance. Were it not for the cohesive group attraction, it would completely destroy the group.

The cohesive force in fish society seems primarily innate, although the influence of training may be in some cases readily demonstrated. In birds, training plays a much greater role, many species form sentiments about others (Bannister, 1932) and acquire social companions among them (Lorenz, 1937). A short exposure of the gray-lag goose at the time of hatching to an observer may cause the goslings to follow him (Heinroth and Heinroth, 1928). Hence, the stimuli calling forth the "herd instinct" may be in one species innate and in another learned. Cowbirds which return to their own flocks after being reared by foster parents apparently have an innate species attraction. In mammals, also, learned or unlearned incentives, such as species odors, may produce the adequate stimulus for calling forth the herd response. In

domestic mammals, such as dogs, the innate attraction may be reduced to a minimum.

The elaborate development of the voice in birds formed an important advance in vertebrate phylogeny. Already at the frog level, voice had important functions both in attracting females and repulsing males (Noble, 1931). It could be used also to warn companions or frighten enemies. But frogs never developed the variety of meaning to their calls found in birds. Suggestive movements more than sounds regulate the social life of fish. They control the movements of the school and correlate the feeding activity of the group (Welty, 1934). The courtship behavior of the female *Hemichromis bimaculatus* will call forth similar behavior in the male (Noble and Kumpf, 1936). When the European minnow, *Phoxinus*, is aroused by the odor of its own cut skin, it dashes away in fright, and this state is apparently transmitted by the rapid escape movements to other members of the school, even if they have not sensed the odor (von Frisch, 1938). In *Tilapia macrocephala*, the same reaction occurs but only in juvenile fish. In birds, movement, posture and especially voice play an infinitely greater role in correlating the activities of the group. If a gull stretches its neck in a particular manner or a duck moves its head in a special way, the other members of the flock prepare at once to fly. Moods are rapidly spread throughout the group by an expressive attitude of any one individual (Lorenz, 1935; Tinbergen, 1936). Some fish, such as *Hemichromis*, brood their eggs and call their young to them with distinctive fin gestures. Further rapid movements of the parents may cause the young to flee. The control of the behavior of young birds by specific cries of their parents was an important advance in vertebrate phylogeny. It made possible greater protection for the young during this critical period of their life history.

In brief, while in the evolution of vertebrates there has been a great improvement in social behavior, at the fish level at least four of the principal components of social



life found in the highest vertebrates had already developed: (1) group attraction, (2) dominance-subordination behavior, (3) suggestion, and (4) parental service. In each of the three classes: fish, birds and mammals, great variation in some of these components occurs. Peck right has repeatedly changed to peck dominance, and parental behavior has dwindled or disappeared. But where these elements of social behavior occur, they are readily recognized in spite of improvement or degeneration.

In view of the repeated occurrence of these elements in different classes of vertebrates, the question remains, are the neural mechanisms responsible for these types of behavior the same in all vertebrates? If we remove the forebrain of a teleost, such as *Hemichromis bimaculatus*, it feeds and swims perfectly, but it can not follow a school (Noble, 1936). This is due to its inability to follow the school in its rapid turnings throughout the tank. The inability of fish to take a variable path may be accurately measured by testing it in a maze. An effective type is an opaque maze with a long path leading from the starting chamber directly to an outer chamber of the tank and arranged with a series of side boxes leading off either side of the central corridor. Intact fish when familiar with the maze will invariably explore the side compartments. No food need be given, since adequate motivation is the tendency of fish to escape from an enclosed space into a better lighted aquarium. *Danio malabaricus* with a mere rudiment of the forebrain remaining differ markedly from the controls in dashing straight down the corridor or at least showing very little tendency to explore. By testing a series of *D. malabaricus* with various forebrain lesions, it was found by Noble and Levin (unpublished) that an area in the caudal third of the corpus striatum was required if the fish were to enter many side boxes. It is this area of the forebrain which is required if the fish are to maintain their schools.

Large lesions in the rostral half of the corpus striatum of *Danio malabaricus* had no effect on the schooling abil-

ity, nor, in *Xiphophorus helleri*, on the dominance-subordination relation of the fish. But deep lesions in the caudal half of the corpus striatum caused a complete loss of all dominance behavior of the species tested. Male *Xiphophorus* with this area destroyed could mate but only when suddenly confronted by a female. In competition even with much smaller males they were at a decided disadvantage. *Hemichromis bimaculatus* with the caudal fifth of the corpus striatum intact can lay or fertilize eggs, but courtship and brooding movements are either entirely absent or very defective. Hence, in both *Xiphophorus* and *Hemichromis* the social relations require that the caudal part of the corpus striatum be present.

It was interesting to find that female *Hemichromis bimaculatus* with broad but shallow lesions of the dorsal surface of the corpus striatum were sometimes able to synchronize with the male during courtship and egg laying but never succeeded in cooperating in the care of the young. The females could not resist attacking other fish, even their mates, which came near their young. This condition persisted throughout the life of the fish. In the best case this was 19 months after the operation. During this period the fish bred 12 times and showed the same defect at each spawning (Noble, 1937). In another group of cichlids (*Heterogramma*, *Apistogramma* and *Nannacara*), the female normally drives the male from the eggs after fertilization. Hence the unbalance caused by superficial lesions of the corpus striatum in one group of cichlids produced a result very similar to the normal pattern in another group.

This does not mean that the operations had changed the brain of *Hemichromis* to resemble that of the dwarf cichlids. The total social pattern is not the mere sum of a number of simpler reflexes but is a new organization with properties and rules inherent in itself. Disturbing the balance by surgical procedures may produce new rules, which in this case were similar to those in another group of fishes. These rules were the same in spite of the fact

that the neural mechanisms available in the brains concerned were very different.

Fish and pigeon, although far removed from one another in the phylogenetic scale, agree in requiring the dorsal portion of the corpus striatum (hyperstriatum of pigeons) for successful integration of the mating and brooding reactions (Rogers, 1922). The movements of spawning may be retained in fish after loss of this area and some of the movements of courtship are present in male pigeons after deeper lesions. Pigeons and other birds differ remarkably from fish, however, in requiring the basal part of the forebrain for independent feeding behavior.

In the mammal, removal of the cortex, a structure not found in teleosts, apparently destroys the dominance behavior. At least Winslow (1938) has described such behavior in laboratory cats, and the behavior of the decorticate cat described by Bard (1934) indicated that it was not respecting the rules of such cat society. In the rat, lesions of the cortex reduce the variability of path selection as measured in a maze (Krechevsky, 1937). Large cortical lesions also disrupt the parental behavior of the rat (Beach, 1937, 1938; Stone, 1938) and also the mating behavior of the male of this species (Lashley, 1938). The rat resembles such viviparous fish as *Xiphophorus* in that the male directs his sex behavior towards both sexes and even other species at puberty (Stone, 1922). Sex recognition in *Xiphophorus* is a matter of learning the color and form of the adequate sex partner. In the rat, it seems that the darting movements of the estrous female are learned in the same manner. In the rabbit, where these cues to sex recognition are not available, apparently olfactory cues are combined with tactile and other sensory data to identify the adequate partner. At least rabbits with cortex removed but still retaining their olfactory tracts are able to copulate (Brooks, 1937). Since the female is the more passive partner, destruction of the olfactory bulbs as well as the cortex fails to destroy her mating

behavior. In brief, in those mammals where the male identifies the opposite sex by its distinctive movements, the destruction of the cortex leads to failure to mate. In birds and fishes, it is the destruction of deeper parts of the forebrain which has this result.

The neural mechanisms which regulate social behavior may be activated by fewer kinds of stimulations than normally activate them. A *Hemichromis* female may identify the sex of another fish of her own species when in an adjacent tank and become paired with it. A male pigeon, isolated from his mate, will produce pigeon milk if he can see her brooding (Patel, 1936). Normally, tactile and other sense modalities function in these social situations. Defects of behavior following lesions of the forebrain are not due to losses in either the sensory or motor systems, but to a disorganization of the neural mechanisms responsible for the behavior. The mechanisms of sexual and parental behavior have periodically their thresholds lowered by hormones. Nevertheless, parental behavior may be produced in fish without hormonal stimulation (Noble, Kumpf and Billings, 1938) and the same is true of mice (Leblond, 1937). If the mechanism is destroyed by removing particular areas of the forebrain, hormones are ineffectual in producing the response.

It is clear from the experiments indicated above that there has been a shift of the brain centers necessary for the adjustment between two or more individuals of a social group, from the corpus striatum of fish and birds to the cortex of mammals. Social drives, such as those of dominance, sexual and parental behavior, are the expressions of the activity of these specific mechanisms (Lashley, 1938). Although the organization of these mechanisms is very inadequately known, the operative work indicates that they have changed their locus in the forebrain during the evolution of the vertebrates. The location of the chief mechanisms of social behavior in the cortex of mammals is correlated with increasing importance of this organ of association in the life of mammals. With the

elaboration of the cortex in the primates, there followed other improvements in social behavior. Tradition became more important than in lower forms and insight into the benefits of cooperation formed an important advance. Many of the old components of social behavior, such as that of dominance, were greatly modified (Maslow, 1937). It is these improved components of social integration that form the basis of human society.

#### SUMMARY

There has been an evolution of the social organization of vertebrates from fish to man. Nevertheless, throughout this series the same components of social behavior may be recognized: (1) group attraction, (2) dominance behavior, (3) parental behavior and (4) suggestion. An improvement in the social organization has included: (1) a change from inborn species attraction to a learned group attraction, (2) from a dominance behavior, recognizing only the individual, to one recognizing groups, and (3) from a subordinate, that considers the dominant individual only as a despot, to one that considers the latter a protector and guide. At the fish level the mood of a member of a social group may be quickly transmitted by the character of the individual's movement to other members of the group. Among higher vertebrates these movements are supplemented by vocal expressions which have specific effects upon the behavior of individuals in the group. In the absence of the forebrain, no social behavior is complete in any vertebrate. Forebrain mechanisms essential for social behavior have shifted from the corpus striatum of fish and birds to the cortex of mammals. The elaboration of the cortex in the higher primates is correlated with an increase in the importance of tradition and insight in regulating social behavior.

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# SOME CONTRIBUTIONS OF THE LABORATORY RODENTS TO OUR UNDERSTANDING OF HUMAN BIOLOGY<sup>1</sup>

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## I.

IN the halcyon days some thirty years ago when the small group of then existing experimental mammal geneticists was intellectually drunk on the bock beer of newly rediscovered Mendelism, the idea of such a topic as that which I am to discuss would not even have entered the genially bewildered heads of a program committee.

It takes the sobering effects of a huge mass of scrambled literature extending over three decades to make biologists begin to consider the possible debt which they, and the generation which they represent, owe to any given type of material. Only after having filled the avid maw of research workers faithfully and well for a long time is the lowly plant or animal given a partnership right to have its name included on the titular signboard of a general paper in a symposium of this sort.

However, to one who still enjoys titillating his long abused and ample nostrils with the more than ebb-tidal aroma of the rodent laboratory, and who still thrills at filling his increasingly carcinophilic lungs with the rat and rabbit-ridden, guinea pig-glutted and mouse-muddled air of that enlivening environment, the challenge of presenting such a paper is a welcome one.

It is amazing how effectively experimental work with laboratory rodents has shaped the development of our whole concept of human biology.

The influence of the comparative point of view has nowhere been more consistent and important in providing

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both a check to too much exuberance and a stimulus to lagging initiative, than in that field.

Let us see, then, whether we can assemble and classify certain facts and impressions bearing on this question in such a way as to help us to evaluate what we already owe and may in the future continue to expect from our scented servants, the laboratory rodents, whose parasites, intestinal and pulmonary infections, abortions and "snuffles" have given us care and sorrow along the road.

We may begin by a brief and admittedly much condensed reference to some of the direct and long-standing contributions which laboratory rodents have made to our knowledge of human biology by serving as invaluable test animals in bacteriology, serology and other phases of experimental medicine as well as in biochemical studies, especially with vitamins and hormones.

Cases of this sort are myriad. To attempt to list them would serve no useful purpose. It is, however, interesting to note that there is more than one way in which the relationship between the human phase of the problem and its laboratory investigation has expressed itself.

There is, for example, the directly comparable situation in which there exists close similarity or identity between the substance studied in rodents and in man. To this group belong the vitamins and hormones. Here the observed results of excess or deficiency are usually directly transferable from the laboratory rodent to man.

Really belonging to this general group also are such procedures as the Friedmann and Aschheim-Zondek tests for pregnancy, although the physiological condition of the human being involved is detectable by the stimulation of a quite different physiological response in the rodent.

Then there is a less direct but still relatively simple relationship in which some microorganism or virus is transferred from man, where it naturally occurs, to rodent material with consistent and predictable effect upon the latter. The studies with yellow fever, syphilis, influenza and pneumonia are cases in point.

In both of these groups there has come during recent years a realization that the genetic nature of the rodent material was an extremely important factor.

Interestingly enough there have been recorded marked genetic differences between inbred strains of rodents, not only in respect to their balance of hormones or of vitamins which are natural elements in their biochemical make-up, but in their reaction to influenza or to yellow fever which are caused by agents utterly outside the experience of the ordinary laboratory rodent.

The discovery of this field of inherited differences in hidden potentialities is a fascinating one, for it suggests that by subjecting animals to a great number of foreign stimuli additional genetic units or complexes will be discovered. In forms, such as mammals, which breed slowly, any method of supplementing the tantalizingly infrequent process of mutation is a welcome weapon for research.

The recognition of the importance of knowing the genetic background of the material used in experimental medicine has spread rapidly. It can largely be credited to the repeated unavoidable and insistent demonstration of this fact by rodent material. This is a great contribution to the advance of human biology. It takes a repeated and varied demonstration of the miracles of the genetic Moses to impress permanently the hard heart of the Pharaoh of the medical profession; and it is necessary to influence that worthy group because by its manipulation of man's ignorance it holds in bondage the application of recognized biological principles to human material.

The emphasis on genetics which results from studies on laboratory rodents is also having a very definite effect, by lifting the fog of misunderstanding and erroneous information which has led the medical profession to speak of "hereditary syphilis" when it meant "transmitted." These two processes, "heredity" and "transmission," have remained in narcotized confusion in the medical mind for decades.

## II

Other facts made patent by experiments with rodents have had a most salutary effect upon the over-enthusiasm of those organizers of post-Mendelian eugenics who, like the fond parents of a child with some dramatic talent, attempted to make their immature offspring something that it was not. The memory of those days when worthy ladies and gentlemen rushed their half-clad infant genes for all sorts of strange physical peculiarities and mental maladjustments, upon the stage of eugenic literature, is still green to many of us. It is, however, the green of the oyster and not of the laurel wreath.

Only the evidence derived from experiments with rodents has prevented the mathematically subdivided man of Galtonian heredity from being reconstructed as a modernistic mosaic Frankenstein of genes for every conceivable characteristic from nystagmus to wanderlust and from hypospadias to insanity,—which is probably a good place to stop.

The rapidly rising tide of recognized complexities in the genetics of rodents has been effective in showing us that what looked like solid ground was dangerous and shifty mud. First, naturally, were experiments that showed exceptions to clear-cut Mendelism—different types of spotting that did not segregate properly; many genes that influenced a single character, many characters that were influenced by a single gene; normal “overlaps,” the living reminders of a widening gap of uncertainty lying between genotype and phenotype. These things showed the danger of too simple a conception of human biology. They even went so far as to begin to divide geneticists into two very distinct groups—those who tried to bind up the wounds which a cruel world had inflicted on the still toddling infant, “human heredity,” and those who walked by (some I regret to say chuckling eerily) on the other side of the street.

Those who chose the latter course might have gone on in smug self-satisfaction had not the brilliant analytical

methods devised by Wright made them suddenly aware that their own armor was not any too sound. It was not only man who presented difficulties. Laboratory rodents were forcing upon man a picture of the complex relationship between genes and development and between intrinsic and extrinsic factors, that has completely made over his approach to his own biological problems.

The need of direct observation to replace pedigrees reconstructed from socio-biological data gathered by eugenic sleuths became admitted and forms to-day a *sine qua non* of research in human biology. When transferred to a problem which lasts for many generations this principle demands patience and permanency of an investigation over a long period of time. The relative importance of any one individual investigator at any one time becomes less, and that of a well-established program of research, more.

The futility of relying upon small numbers of individuals impresses itself more and more upon the investigator. The difficulties encountered even with the easily recorded, readily measured characters in abundantly prolific laboratory rodents say clearly to the human biologists "Stop, Look and Listen" before crossing the railroad track which divides speculation from accepted proof.

### III

But it may be felt that all that laboratory rodents have contributed to the better understanding of human biology is confined to our digging, with hard facts and difficulties, the grave in which the fruits of the premature conceptions of that subject are buried. This is far from being the case.

There are two levels on which the experimental results obtained with laboratory rodents influence most vitally and constructively the course of development of some of the most important principles of biology as applied to human beings.

The first of these levels is that provided by the fact that

the life span of laboratory rodents is of the right length to present an opportunity for continuous study of the successive chemical, morphological and physiological stages which characterize the history of the individual mammal from conception to death. This is a definite and concrete contribution to the solution of problems of human biology.

The second level is less easy of definition. It is that of the effect which the facts derived from a study of rodents have upon the socio-biological philosophy of our generation. Very evidently in spite of its somewhat intangible quality this influence of laboratory rodents may, if constructive, be broad in extent and of great lasting importance.

Let us first return for a short time to the consideration of the more definite and central ring of influence—that of the convenient life cycle—before taking up the wider ripples which lap against more speculative shores.

The existence of a considerable number of different genetic strains of laboratory rodents, notably mice, is the result of a program of intensive inbreeding, sufficiently prolonged to have produced within each strain a high degree of genetic homogeneity.

Within any such inbred strain the transplantation of tissue, either normal or tumorous, is ordinarily as successful as is transplantation of a bit of similar tissue from one part of an individual's body to another. On the other hand, successful transplantation outside of the inbred strain is ordinarily much less frequent and is often entirely impossible. The rate at which individuals of a certain strain eliminate the foreign tissue placed in them varies according to several factors. Chief among these is the age of the individual receiving the implant. The foreign tissue persists for a longer time in very young animals and in very old animals of an inbred strain than it does in young adult animals. Translated into general terms this means that the full expression of the genetic characteristics of the strain, which enables it to recognize the implanted tissue as foreign, is gradually acquired and, as senility comes on, is gradually lost.

Similarly, if *variation* in a given physiological function is studied at different ages of the individual it will prove to be greater when the individual is at the age when the function begins, less as the individual approaches the peak of its physiologic efficiency at the young adult stage and then greater again in old age.

These two lines of evidence contribute very importantly to our understanding of mammalian individuality—a term at once both essential and difficult of definition. We are perhaps too prone to focus our attention upon the relatively clear morphological limits within which an individual mammal is confined. This gives us a false sense of definiteness and of permanency. The truth of the matter is that the characteristic attributes of mammalian individuality are its reactions and physiological potentialities at any given time. These reflect much more accurately the qualities which make the animal typical of a given strain or species than do the variations in morphology which cater to our most subtly deceptive sense—vision.

From a large number of observations on laboratory rodents we can conclude that the nature of mammalian individuality is that of a complex process which has a durational phase and that it is the product of a balanced and relatively constant internal environment gradually acquired, retained for a time and then gradually lost.

#### IV

The bearing of this conclusion upon the study of human biology is very evident. Chronological age in mixed genetic material at once loses much of its significance as a factor of individual importance unless the degree to which relative elements and characteristics have developed is also taken into account.

This of necessity drives us back to investigation of the phenomena of experimental embryology, experimental morphology and physiology so that ontogenetic correlations between various structures and/or functions can be

detected. By investigation of this kind with known genetic strains of laboratory rodents with short life cycles it may be possible to shed some light upon the now almost impenetrable darkness of man's internal environment, as it stretches from the fertilized ovum through adolescence and adult life to senility and death. And though there may be delay and difficulty involved in such investigation, we can confidently say that without the use of laboratory rodents to give us the main points of importance applicable to the long and involved life cycle of man, we shall not succeed in solving the problem.

The vast importance of the short life cycle of laboratory rodents is nowhere more evident than in studies of the so-called degenerative diseases, which really mean those conditions of unbalance in the internal environment which indicate that centralized control of function has begun to break down.

Among these cancer is perhaps the classical example. At all events, it has held the center of the stage of public interest for some time because of its growing menace as a cause of death. The contribution made by laboratory rodents to our knowledge of the various manifestations of uncontrolled growth (known as cancer) is an impressive one. It includes data based on the rate of incidence of uncontrolled growth of various types of tissue in different inbred strains. These data have once and for all enabled us to state that the causes leading up to a change from normal to cancerous tissue are multiple and very varied in type.

This line of research also definitely links ovarian hormones and perhaps others as contributing factors which influence the incidence of cancer of the breast. Here, by holding genetics constant and varying the degree of function of internal secretions, and then by reversing this process, we have been able to make real progress in our understanding of how both genes and endocrine glands may interact to produce a given end result. It further emphasizes the glaring defects in our knowledge of the

period attendant upon the mammal's change from intra-uterine to post-natal life. There is evidence which suggests that in mammals this may be a period of a far more critical degree of biologic elasticity and adaptability than we have, up to now, appreciated. This line of research also gives us new and convenient indicators of physiological longevity and in fact opens the door to a whole host of different and fascinating avenues of attack on problems basic to our understanding of man as a mammal in relation to his own complicated internal environment.

## V

One might continue indefinitely the discussion of the contributions which the laboratory rodents have made and will undoubtedly continue to make in this field. Since, however, time is limited, it may be well to give an example or two of the less tangible but not less important contributions which laboratory rodents have indirectly made towards the development of a sane attitude in human biology.

The first of these that I shall mention has a certain lighter side. We are all aware of the emotional bubbling and irrational babbling of that strange fellowship of the anti-vivisectionists which pours in a stream over the paralyzed or bewildered brains of legislators at every opportunity and which in such sun-struck states as California has recently become a menace to scientific research. What we do not perhaps realize, however, is that within the ranks of that sturdy body it has been difficult to keep at fever heat a level of sympathy for the rodent similar to that which the dog or cat engenders. Were it not for the appearance of rabbits upon Easter cards and its resultant heart throb, the rodents would be a completely constructive element. The age-old enmity of woman and the Muridae may yet save the day. Seriously, however, the division of forces and the resulting restriction of objective among the anti-vivisectionist, caused by the cold and impersonal eye of rat, mouse, guinea pig or rabbit, is a real



contribution. It may make the rank and file of citizens understand more clearly the abnormal nature of the unbalanced and starved emotions that lie at the base of organized attempts to destroy scientific research with mammals. Since without that research human biology is doomed, the issue at stake is a truly great one.

Another intensely interesting field in which research work with laboratory rodents has exerted an important influence on human biology is that of experimental modification of the germ-plasm. When in 1923 the writer and Bagg published evidence of a genetic modification following the treatment of mouse ovaries with x-rays that agent was being extensively used to correct irregularities in the human menstrual cycle. Little attention was being paid to the subsequent reproductive history of a woman so treated, although certain medical investigators believed that they had evidence of an increased proportion of monsters among children produced following treatment. There is, however, clear evidence that to-day—fifteen years later—a great deal more caution is being exercised. This is because of the fact that although the radiologist applying the rays may not be concerned directly with abnormalities that may crop out in a second or third generation it is recognized that *some one* has to deal with them eventually, once the germ cell change has occurred.

Similarly, the incidence of damaged progeny resulting from injured germ cells which can still fertilize or be fertilized, has been demonstrated in rodents. This fact has or should have a permanent effect upon those who advocate research in contraception by any technique which kills germ cells—unless—and it is an important condition—the treatment kills *all* germ cells and does not include the possibility that some may be injured and still function.

Here then, by making laymen and the medical profession assume responsibility, not only for the generation produced under their immediate supervision, but for those which in the future may bring out hidden abnormalities resulting from ancestral injuries, the laboratory rodents

have performed a very great and impressive service. They have awakened a new sense of the importance of building for the distant future as well as for the present.

There is, moreover, another way in which the laboratory rodents have done a great and lasting service to those of mankind who are not to-day blinded by emotion disguised as pseudo-science. They have made racial "superiority" and "inferiority" antiquated terms in human biology. They have done this in various ways but chiefly by giving us living examples of the unsoundness of many of our former ideas and of the terms which we very glibly used to describe them.

By providing overwhelming evidence of the complex genetic situation in "strains" of laboratory rodents, the implied value of such terms as "race," "strain" and "family" among the vastly more complicated human "genetic" groups has disappeared. When it was obviously none too easy to find any satisfactory criteria of qualitative differences between *species* of laboratory rodents it was impossible to become as aroused as formerly over supposed "racial" or "national" superiority-differences between human beings of the same species.

The philosophy which thus arose from the evidence obtained in the rodent laboratory is one which very definitely relegates the troublesome and stupid distinctions which agitate the world to-day to the realm of propaganda without scientific basis.

We know that we can, under different environmental conditions in the laboratory, develop by selection strains which differ genetically from one another so that very dissimilar end results are obtained. The superiority and inferiority of these end products is, however, a matter which depends entirely upon the circumstances in which they are placed. There is no doubt that to one type the others may be pleasant or unpleasant, welcome or unwelcome. That these reactions are determined by a fixed and ingrained biological superiority or that, between a given characteristic and its basic value, there exists an anthropological

and age-old relationship is, however, entirely unsupported by scientific evidence.

And if in answer to this those who still insist that we must have distinctions appeal to man's psychological and social inheritance as of prime importance, the ample and insistent evidence derived from the humble laboratory rodents leads us back, even though we be blinded, to the conclusion that neglect of biological foundations can and will upset the best-laid plans of sociological reformers or eloquent demagogues.

In a world in turmoil because of unsatisfied greed it is not a bad idea to seek refuge in research concerning man and more especially his inner biological nature. We shall find that his worst enemies still lie there. He is still bound by the very nature of his biological organization in a tangled net of little-understood phenomena. I suspect that research with laboratory rodents will be in the future, as it has been in the past, the chief means of breaking one by one the strands of that net. I suspect that the gnawing of the teeth of mice, rats, guinea pigs and rabbits upon the bonds of man's ignorance will be heard long after dictators stop their bellowings, long after armed strength is paraded for the last time across the great squares of man's stone-blind stupidity and long after we in America stop worrying about the idea of democracy and make it an actuality.

# THE USE OF THE MONKEY AND APE IN THE STUDIES OF HUMAN BIOLOGY, WITH SPECIAL REFERENCE TO PRIMATE AFFINITIES<sup>1</sup>

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THE chief subject for the study of man will always be man himself. For the discovery of fundamental principles applicable also to man, however, it may be and has been in the past *Ascaris* or *Drosophila* or the white rat. Where lower animals are suited to answer our questions and help solve our problems it is extravagant, often undesirable for other reasons, to use the monkey. Because of the limitations of experimentation on the human being, however, in order to bridge the gap from the lower forms to man, primate animals must be the subject of choice. For three lines of investigation, apes and monkeys would seem to be indispensable: the study of certain diseases; the origin and affinities of man; problems of reproduction, notably menstruation. One might add problems of neurology; but I prefer to discuss these briefly under the second head. Of the first I know little; in the second field some of my friends have over a period of years attempted to orient me; in the third I hope to make a small contribution to the subject through first-hand information and to show how some recently acquired knowledge of reproductive processes in apes and monkeys substantiate the general conclusions that have previously been drawn on morphological evidence.

We may dismiss the subject of special susceptibility of monkeys to certain human diseases with the remark that this is just another evidence of the close affinity of monkeys to man.

<sup>1</sup> Read at a Symposium of the American Society of Naturalists in joint session with the American Society of Zoologists, the Botanical Society of America and Section H, The American Association for the Advancement of Science, Richmond, Virginia, December 30, 1938.

In attempting to gain a bird's-eye view of the chief lines of evidence bearing upon man's place in the order of primates we become aware first and foremost of an array of studies on the configuration of (a) the teeth; (b) the skeleton. This preponderance of data on the hard parts of organisms is due partly to the fact that skins and skeletons alone are suitable for convenient transportation from the field; the perishable soft parts usually have of necessity to be left behind. Furthermore, the soft parts do not lend themselves as readily to metric analysis and among higher primates differ qualitatively even less than the hard parts. Since dental and skeletal measurements have the advantage that great accuracy is attainable, the data may be plotted on curves for comparisons from species to species.

A perusal of the literature discloses the fact that several generalizations may be made without arousing too much opposition from one quarter or another (*cf.* Wool-lard, 1938).

First, there exists to-day no "contemporary ancestor" of man—no primate in the process of becoming human. In other words, each species occupies an end-twig of the family tree and each group has differentiated in its own way since leaving the ancestral stem. Concerning the comparatively few fossil primates thus far unearthed, there is no agreement as yet among paleontologists as to whether these constitute true "missing links" in the direct line of descent or mere side-branches on a par with the 600 living species of primates.

A second conclusion seems imperative, that a valid seriation of primate groups on the basis of degree of resemblance to man or to chimpanzee or to the rhesus monkey must consider a great many characters. As Schultz has repeatedly shown, an arrangement in respect to the degree of development of one character may or may not hold for another character. If the characters are weighted for degree of importance and the data treated statistically one finds in general that the anthropoids re-

seemble man much more than do lower monkeys; that catarrhines (Old World monkeys) form a group nearer to man than the platyrrhines (New World monkeys); that chimpanzee and gorilla form a closely related group somewhat distinct from the orang-utan and still farther removed from the Hylobatidæ (gibbon, siamang). Taking the most studied chimpanzee as an example of the anthropoids and the rhesus monkey as the most studied catarrhine it is true that in many characters the chimpanzee is more or less intermediate between monkey and man. We shall a little later add several new items that fall in line with this thesis.

While man has differentiated in his own way in numerous characters we appropriately call "human," he also possesses characters which may be designated as primitive, inasmuch as, according to Straus, they resemble those of the platyrrhines rather than the apes or higher monkeys. It would seem, therefore, that he deviated from the ancestral stem in early geological time, since he carries those primitive characters. The family tree of Weinert (Fig. 1) must be wrong, that of Schultz and that of Gregory more nearly in line with the facts.

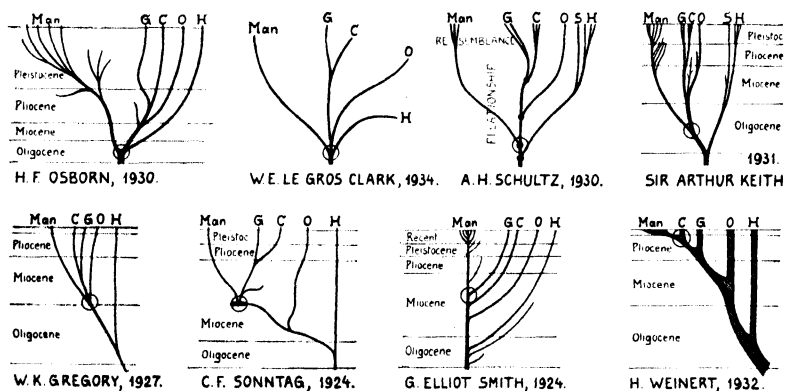


FIG. 1. The family tree of the higher primates according to different authorities. G, gorilla; C, chimpanzee; O, orang-utan; S, siamang; H, gibbon or Hylobatidæ in general. From Schultz, 1936.

A final conclusion is impressed upon the non-specialist reading in this field of anthropological study, namely, that

the science of primatology has but begun. Not only must we know something of many more species, but we must know a great deal more about the few thus far investigated on inadequate material.

If this be true of the more accessible hard parts of the organism what hiatuses do we not see in respect to our knowledge of the growth and variations of the soft parts? These have not as yet entered much into the calculations of either the taxonomist or the anthropologist, since data from one or two specimens of each species have very limited significance and since adequate series of properly preserved specimens are rarely obtainable and few investigators have the infinite patience needed for dissecting them. The growth of the skeleton depends, indeed, upon the action of soft parts: anterior pituitary, parathyroid, gonads, all under the final aegis, of course, of the genetic constitution. As an example of a study of soft parts checking and corroborating that of skeletal parts and teeth, the rabbits and hares might be cited, which, on the basis of skeleton and teeth, Gidley removed from the *Rodentia* and created for them a new Order, the *Lagomorpha*. One thinks at once of certain other characters that argue strongly in favor of Gidley's contention: (1) the albumen-covered ovum, (2) the interstitial cell-laden ovary, and (3) the comparatively simple method of early development and implantation of the rabbit as compared with all the known *Rodentia* (Hartman, 1925).

The many hiatuses in our knowledge of primate morphology, until corrected, will continue to stimulate absurd interpretations of human structures. It is too easy to say: "Not found in any subhuman primate"—which may be correct but is misleading, for the point at issue may not have been investigated. To cite an example: In our youth we were taught that because man possesses an opposable thumb (barring the lowly opossum, which apparently did not count), he is also the only being provided with the appropriate muscle (*M. opponens pollicis*). The hard fact stands, however, that all species of apes, monkeys and even lemurs thus far studied possess this muscle, though

its size may be variable and the proportion of individuals within the species in which it is absent varies from species to species. Moreover, the opposability of the thumb is a primate character and, as Schultz has shown, the species as a matter of fact differ only in degree of opposability.

Next to the skeleton it is the primate musculature which has received the greatest amount of attention, first by the Swiss anatomist, G. Ruge, whose mantle fell upon the late E. Huber, who brought to the Johns Hopkins Ruge's spirit and techniques. Huber's work on the comparative anatomy of the facial musculature and its innervation; likewise that of Howell and Straus on the general musculature of the rhesus monkey, accompanied by comparative notes, are outstanding in their extent and thoroughness. For seriation of the primates these studies have, however, led to discordant results. Thus the human hand is primitive as compared with that of the chimpanzee, and more like that of lower primates. In consonance with this conclusion we see that an infant when it walks on all fours lays the hands down flat like a rhesus monkey, not flexed, with knuckles on the ground, like chimpanzee or gorilla (Hrdlička). On the basis of his studies on the viscera also Straus finds that man has a greater similarity to the more primitive Old World monkeys than to the anthropoids.

Compared with primate anatomy our ignorance of primate physiology is even deeper and wider, as has been shown by Zuckerman in his interesting book, "Functional Affinities of Man, Monkeys and Apes." These gaps should be filled, for it is true that "every structure and property of the animal or plant, whether they be organs or cytological structures, physiological activity, habits or ecological relationships," constitute characters of potential value for taxonomy or phylogeny.

Thus far physiological data of phylogenetic value have come from three main lines of investigation: (1) Blood groupings and agglutination phenomena; (2) physiology of the nervous system; (3) physiology of reproduction.

The precipitin reaction already in the hands of Nuttall



gave quantitative indications of the relationship of the primates. In these experiments the anthropoids approached nearest man, the platyrrhines appeared furthest removed. For more accurate because more delicate tests we turn to the experiments of Landsteiner and Miller (1925), Mollison and others, who worked with blood-group specific isoagglutinins. Since each blood-group has its specific agglutinin, a rabbit may be immunized to a particular agglutinin and its serum then tested against the corresponding class of blood in the species studied. Landsteiner and Miller and those who repeated and extended their observations have concluded in general that the blood of the anthropoid apes and that of man are practically identical by these tests and that the Hylobatidae, the catarrhines and the platyrrhines form sharply separated groups, each, however, closely related *inter se*. Other immunological phenomena, on the other hand, give results inconsistent with the seriation thus outlined. While it is evident that too few tests have been made on too few species, largely because of the lack of available specimens, on the whole the blood tests show the great apes intermediate between man and monkey but much closer to man.

Neurological investigations, the most extensive and varied of which we owe Fulton, corroborate this conclusion: In brain size as well as cyto-architecture the anthropoids are far removed from all monkeys, approaching man, though never reaching him even in absolute brain weight. Of particular interest is the Babinski reflex thus far observed only in man and chimpanzee.

The rapid advance in the field of reproductive physiology in the last several decades has been reflected in a considerable growth of our knowledge of reproductive processes in the primates. The availability and hardiness of the rhesus monkey has made this species the chief laboratory primate. The groundwork on the chimpanzee has been accomplished by the Yale Laboratory of Primate Biology, so that this rare, huge man-ape has taken its place beside the white rat, guinea pig and rhesus monkey. In

the near future we may look to the Columbia University project in Puerto Rico for equivalent knowledge concerning the gibbon. The platyrrhines of the Old World still remain almost totally *terra incognita*.

For the experimental study of menstruation the monkey is absolutely indispensable, since only the higher primates exhibit this phenomenon either spontaneously or in response to hormonal treatment. If one injects the female sex hormone into castrated rabbit doe and castrated female monkey, the uterus in either case will grow for a time during the administration of the hormone and in either case, after cessation of injections, the uterus will rapidly regress. The monkey uterus will bleed (menstruate) in characteristic fashion, the rabbit uterus will not. The two species are as distinct with reference to this character as to any other character, for example, their vision, the monkey having stereoscopic vision with overlapping fields, while the rabbit sees an object out of one eye at a time.

Anatomically, the difference in the two uteri lies in the peculiar spiral arteries of the monkey endometrium. In the physiology of these arteries, universal among primates, lies the mystery of the menstrual flow.

So far as known, the uteri of man, apes and catarrhine monkeys have all essentials in common. Only the little-known platyrrhines may be expected to yield some exceptional traits. We already know that their uterine glands are tremendously developed and active (Table 1).

Because of the similarity of primate uteri and the associated menstrual process, experimental findings in the monkey may be directly applied to women, as clinicians are beginning to realize. The rhesus monkey, therefore, occupies a key position for the experimental probing into the cause of menstruation. It follows from these remarks, however, that the study of menstruation will yield little of phylogenetic value within the group, at least until we know more about the platyrrhines. In point of length of the cycle Yerkes and Elder have found the chimpanzee cycle to average at least a week longer than the average

TABLE 1  
PLACENTAL EVIDENCE OF PRIMATE RELATIONSHIPS

Character	Man	Anthropoids (Chimpanzee)	Catarrhines (Macaques)	Platyrrhines (Howler, Spider)	Tarsius
Implantation:	Interstitial	Interstitial	Superficial	Very superficial	Superficial
Decidua capsularis	Yes	Yes	No	No	No
Villi	On all sides	On all sides	Partly lacking	Partly lacking	Partly lacking
Villi formation	Precocious	Precocious	Precocious	Delayed	Precocious
Lacunae in trophoblast	Simple	Simple	Simple	Labyrinthine	Prob. Simple
Decidual reaction	Marked	Marked	Moderate	Moderate	Prob. Moderate
Epithelial plaques	Absent	Absent	Thick, localized	Thin, extensive long persisting	Thick, localized
Definitive placenta	Human or "villous" type	Human or "villous" type	Human or "villous" type	Trabeculated, Labyrinthine	Trabeculated, Labyrinthine
Uterine glands	Single	Single	Mostly double	Double or single	Considerably developed
	Moderately developed	Moderately developed	Moderately developed	Markedly dev. and active	

of a lunar ("menstrual") month, obtaining in women and the catarrhines (Fig. 2). In consonance with the long

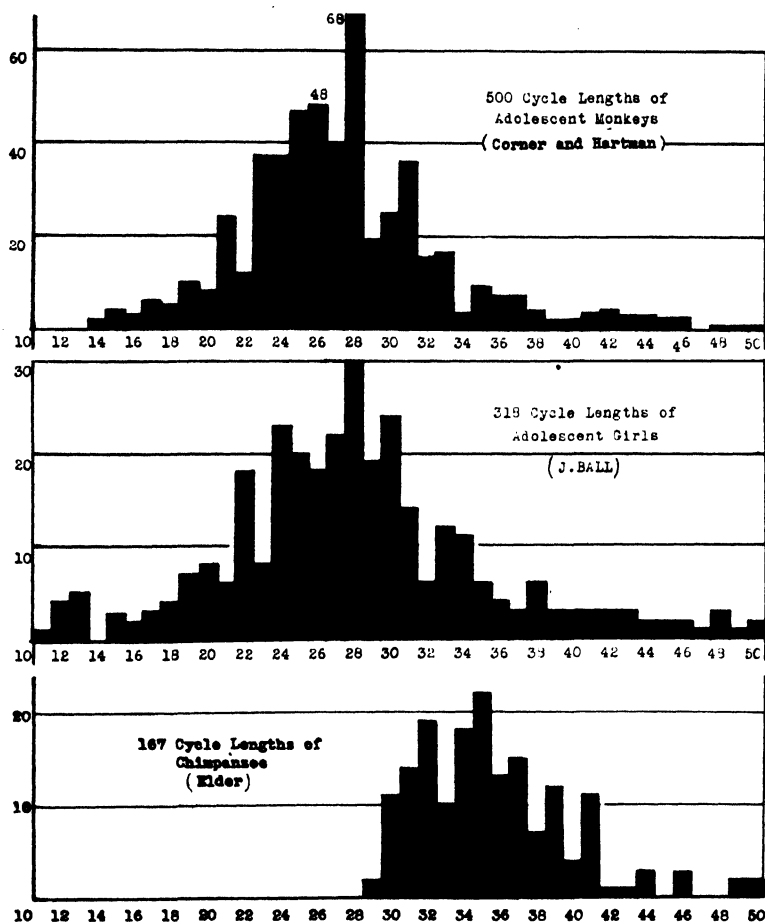


FIG. 2. Graphs of cycle lengths of young monkeys, girls and mostly adult chimpanzees. The graphs for women and adult monkeys would show a little less spread but the same average.

cycle, ovulation in the chimpanzee is also delayed by a week. Hamlett has adduced evidence that the platyrrhines experience two complete cycles in a month. If this is corroborated we have two groups that have differentiated in their own way with reference to the menstrual cycle.

The definitive placenta offers little ground for seriation of the primate groups, except to separate the platyrrhines from the higher members. For, while the primate placenta is sharply differentiated from that of all other mammals, the human, anthropoid and catarrhine placentae present a common type *inter se*. It is in the main only in the early stages that it is possible to differentiate them.

The early development of the placenta is adequately known, from its beginnings, for only one primate species, the rhesus monkey. Based upon a complete series of accurately timed specimens furnished by the Carnegie colony, this chapter in primate biology has been written within the year (Wislocki and Streeter, 1938). The specimens are perfectly preserved and impeccably prepared by my colleague, Dr. Heuser. Human material is next in order of completeness, though the earliest embedding of the ovum, covering a period of two days, is absolutely blank and the condition of specimens beyond the eleventh day (age of the youngest human ovum, the "Miller") often leaves much to be desired. Aside from these two series the 600 species of primates to this good day are represented by only a score of fairly young embryos and implantation sites (gibbon, catarrhines, platyrrhines, Tarsius and the lemurs). By a fortunate set of circumstances we are able to-day to present a 10½-day chimpanzee ovum, furnished by the laboratory of our honorable president, Dr. R. M. Yerkes. Further reference to this beautiful specimen will be made presently.

The features of placentation in primates which seem to me most significant from a comparative standpoint have been condensed in the accompanying table. In the preparation of this I have drawn upon the important monograph of Hill (Croonian Lecture, 1932), that of Wislocki and Streeter (1938) on the rhesus monkey as well as the extensive materials of the Carnegie Laboratory of Embryology. We may restate the contents of the table as follows:

While all primates (excepting the lemurs and Prosimiae) exhibit the burrowing type of placentation, only

in anthropoids (including the gibbon) and man does the vesicle burrow completely beneath the uterine epithelium. This has been known for the orang-utan and the gibbon (Selenka, 1899) because their implantation sites exhibit a decidua capsularis, the more superficial portion of the placenta where villi are imperfectly formed. No early stages were, however, known for any anthropoid until the discovery of the 10½-day chimpanzee which we are able to present to-day (Fig. 3, B). This specimen is about a half-day younger than the youngest captive human (Fig. 3, A).



FIG. 3. (A) The "Miller" ovum, the youngest normal human embryo thus far known. From Streeter, 1926. (B) Chimpanzee ovum, "Yerkes A," the youngest known anthropoid ovum. From Elder, Hartman and Heuser, 1938.

The illustrations show the similarity between the two, which is as complete as imaginable, considering that the chimpanzee is a little younger and smaller and much better prepared than the Miller ovum, which was accidentally found amidst the debris of curettings. The cardinal point is that at 10½ days the anthropoid vesicle is already surrounded on all sides by maternal tissue.

Already in the youngest anthropoid and the youngest human ovum the trophoblastic shell has developed lacunae, although in these there is as yet no extravasated maternal blood. These lacunae enlarge into irregular spaces which

remain fairly simple in all Simiae except the platyrrhines in which the structure becomes very complex by virtue of a profuse growth of trophoblastic trabeculae. Since the condition is also simple in *Tarsius* we may consider the complex nature of the platyrrhine trophoblast as a unique specialization.

A decidual reaction, that is, enlargement and succulence of stromal connective tissue cells, is most pronounced in man and the anthropoid apes. The reaction is much less marked in the rhesus preparations in our possession. But the most striking character, sharply differentiating the lower monkeys from the man-anthropoid group, is the reaction of the uterine epithelium to the "sting" of the ovum. In the vicinity of the points where the 9-day vesicle touches the epithelium this proliferates and thickens, forming thickened epithelial cushions or weals, one on the ventral surface, usually the primary one, and one on the dorsal surface (Fig. 4). These epithelial plaques are con-

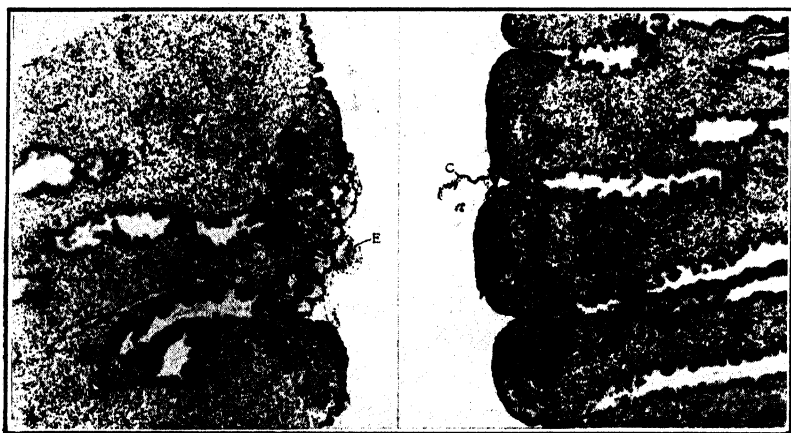


FIG. 4. Sections through endometrium of rhesus monkey C530, showing 12-day embryo (E) and the primary (left) and secondary placental plaques. C, chorion torn loose. From Wislocki and Streeter, 1938.

sumed by the invading trophoblast and form the pabulum of the ovum for about 10 days. No plaques are produced in the human or anthropoid uterus, but in all monkeys and in *Tarsius*. The placental plaque in the catarrhines and

in *Tarsius* is thick and localized, in the platyrrhines, thin, extensive and more persistent. While this again breaks the linear series—*Tarsius*→*Platyrrhines*→*Catarrhines*→*Anthropoids*→*Man*—the character does once more sharply set man and the anthropoids off from the remaining primates.

The characteristics of the very young embryo proper can not yet be utilized for purposes of primate phylogeny because of the meager data at hand. Incidentally it might be mentioned that the four free vesicles included in the collection of rhesus ova conform strictly to the mammalian type. We can confidently conclude that the bilaminar vesicle of man and the anthropoids will eventually be found to conform likewise.

On later prenatal and postnatal growth of the primate data are in hand for man, the rhesus monkey and to some extent for the chimpanzee. The first adequate human series for the study of prenatal growth we owe to Streeter and Schultz. Seven hundred fetuses of the Carnegie collection, mostly from pregnancies with fairly good menstrual records, formed the basis for these studies. Moreover, since each investigator personally measured all the specimens the variable personal equation inherent in compilations from numerous sources was eliminated.

To date, the story of the growth of but one primate animal, the rhesus monkey, has been written. At the Carnegie Laboratory we were able to assemble a goodly number of fetuses and young of this species, concerning all of which the conception age was accurately known, mostly to a day. We were fortunate in having available an interested anthropologist, A. H. Schultz, who measured the specimens and interpreted the observations and measurements in the light of human development (Schultz, 1937). In the accompanying chart (Fig. 5) the curves of intra-uterine growth of man and rhesus monkey are shown. It is seen that the monkey fetus grows a little faster than the human fetus until the twentieth week of gestation, when it is overtaken by the latter. From the data furnished by



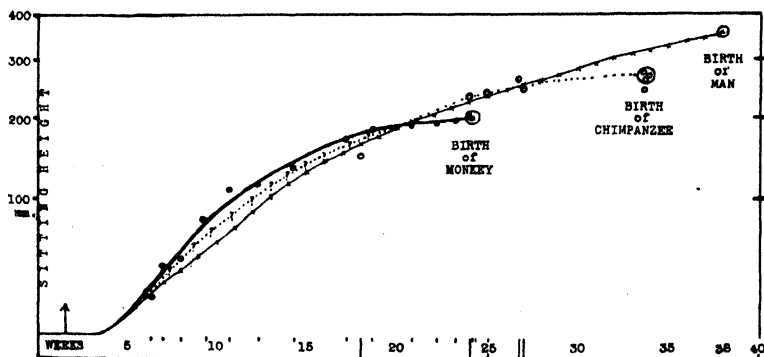


FIG. 5. Growth curves for man (after Streeter, 1920), rhesus monkey (after Schultz, 1937) and chimpanzee (data contributed by A. H. Schultz and Yale Laboratory of Primate Biology). The greater part of the growth curve of the chimpanzee is conjectural, being based on the few cases plotted in open circles. Arrow indicates age of youngest human and youngest anthropoid embryos shown in Fig. 3.

five late fetuses and several new-born babies of the chimpanzee (two cases of which we owe to Schultz, the remainder to the Yale Laboratory of Primate Biology) it seems highly probable that the prenatal growth curve of this species will be found to fall between that of monkey and man and that its growth curve will cross the other two at the 20-week point. We are fairly safe in concluding that at 20 weeks' development the fetuses of monkey, chimpanzee and man are of approximately equal size. We have noted above that the placentae of the three species are so much alike that microscopically they are not distinguishable one from another; hence it is fair to say that a given nutritive tissue, the placenta, is capable in a given time of producing a uniform bulk of baby.

The rhesus monkey is born soon after reaching this interesting point of development. The human baby is larger at birth because it remains longer *in utero*. The chimpanzee is intermediate in this respect. It is also intermediate as to degree of maturity at birth between the very helpless human baby and the monkey baby capable of clinging safely to the mother's body five minutes after parturition.

The chart (Fig. 5) likewise shows the period of gesta-

tion of the chimpanzee (235 days) to be intermediate between the monkey (165 days) and man (267 days). So far as we know the catarrhines in general conform closely to the figures first worked out at the Carnegie colony on the rhesus monkey, namely, 6 lunar months against 10 for man.

To the phylogenetic series—monkey→chimpanzee→man—we are able to add one more functional character. Since Aschheim and Zondek 10 years ago discovered a gonadotropic principle, prolan, in the urine of pregnant women, a search has been made for this principle in the urine of pregnant animals. The results were negative in all but the primates, although in some cases the blood of lower mammals, *e.g.*, the horse, was found to contain enormous quantities of a similar substance. In women it was found that early in pregnancy, around the sixtieth day, prolan is present in the urine in huge quantities, dropping off rapidly to a low titre maintained throughout the remainder of gestation. At the Yale Laboratory of Primate Biology,

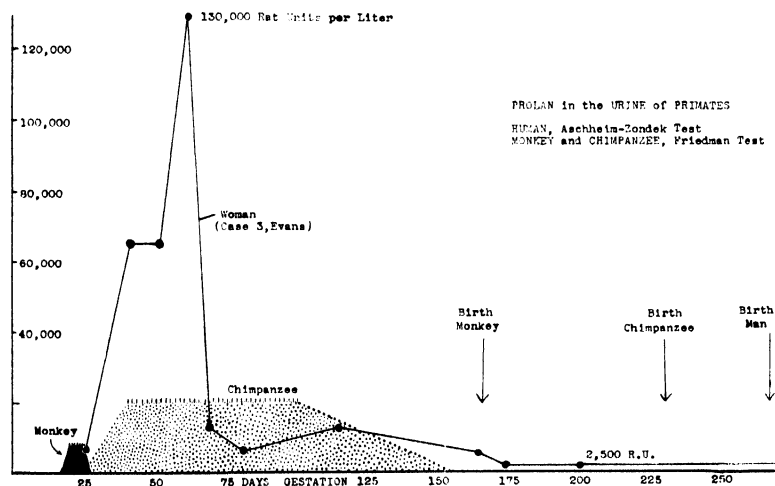


FIG. 6. Chart showing days of gestation in which prolactin (gonadotropic hormone) is present in the urine of rhesus monkey, chimpanzee and woman. The curve for woman was determined on rats and is quantitative; those for monkey and chimpanzee by the rabbit test and are not quantitative. Monkey data from Hamlett, 1936; chimpanzee data (from Elder, unpublished) of the Yale Laboratory of Primate Biology.

Elder was able to recover prolactin from the pregnant chimpanzee as early as the 25th to the 35th day and found it to disappear completely between the 100th and the 160th day. Finally, Hamlett, examining pregnant rhesus monkeys of the Carnegie Colony, discovered that in this species the prolactin output was most evanescent, lasting only from the 19th to the 25th day after fertilization. These facts are recorded in the accompanying chart which graphically represents again the phylogenetic series—monkey→chimpanzee→man (Fig. 6).

Nothing has been said as yet concerning behavioral characteristics and relative intelligence. Fortunately we have Dr. Yerkes to discuss this subject.

Weighing all the facts it is safe to conclude that the primates are separable into definite groups distinctive with reference to many characters: anatomical, embryological, physiological, chemical, hormonal. Seriation of groups in respect to degree of resemblance to man varies from one character to another, but on the whole the series runs roughly: (1) man, (2) chimpanzee and gorilla with orang-utan trailing; (3) gibbon and siamang; (4) the catarrhines; (5) the platyrrhines; (6) *Tarsius*. Certainly the great bulk of the data, to which we have been able to add several new items, points to close affinity of the apes to man as compared with the monkey.

The studies here sketchily reviewed in this lecture point, however, to one incontrovertible conclusion, namely, the fragmentariness and meagerness of our knowledge of primates. The attention given primates is but a tiny proportion of that accorded the laboratory and domestic mammals. More projects like the Yale Anthropoid Colony and the Columbia Gibbon Colony should be initiated, especially one for the study of the American monkeys. We need more men like Schultz to exploit the museums, like Zuckerman to make intelligent use of primate collections in zoological gardens, or like Carpenter to go to the animals in their native haunts and live with them long enough really to learn something about them. I mean this

address to be a plea for a more adequate prosecution of primate studies, and I have attempted to show that in this field we have only scratched the surface.

Fully to understand man we must learn all about man's nearest relations (the primates), so that we can finally show what morphological, physiological and psychological features are really peculiar to man and what others man shares with the apes. We must continue to gather facts diligently and carefully. Once the facts have been ascertained, theory, the most useful guide to fact-gathering, will itself evolve and guide us to the truth.

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# THE SEX RATIO IN WILD BIRDS

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UNEQUAL sex ratios in vertebrates have been generally ignored during the last two decades, although they are of the greatest theoretical importance. Only such cases as could be explained on a hormonal basis have been discussed in greater detail. Professor Crew points out in a recent paper (1937) what was generally known but never sufficiently emphasized, that in vertebrates there is an amazing number of exceptions to an expected sex ratio of 50 per cent. males and 50 per cent. females. He concluded that the classical theory of inheritance of sex will have to be modified in several respects in order to comply with the known facts. Unfortunately Crew did not utilize all the available evidence, with the result that his conclusions, though basically correct, are too specialized, as I shall show below. I shall restrict my remarks almost exclusively to birds, since this order of vertebrates was particularly neglected by Crew, who states: "In the case of birds there is much that is anecdotal and a certain amount of information that has been derived from experimentation." This statement implies that Crew considers all the observations of the field naturalists as "anecdotal" and therefore as unreliable. It is easily understandable how Crew could have arrived at such an opinion, since there is no recent survey of the field available. On the other hand, there actually exists an enormous amount of highly valuable evidence which is quite inaccessible to the laboratory biologist because it is scattered through hundreds of volumes of ornithological journals. I have attempted to summarize this vast literature with particular emphasis on such evidence as indicates the occurrence of unusual sex ratios in birds.

## PRIMARY AND SECONDARY SEX RATIO

It is convenient for practical purposes to classify observed sex ratios in three groups. The *primary sex ratio*

relates to the proportion of the sexes at the time of fertilization, the *secondary sex ratio* at the time of birth and the *tertiary sex ratio* during adult life.

The three sex ratios will remain identical, if the death-rate of males and females during prenatal and postnatal life runs exactly parallel. It has, however, been found in most animals that the death-rates of males and females differ at every period of life. This difference may be caused by genetic factors (sex-linked lethals) or it may be due to physiological factors (different rates of metabolism) or to environmental factors (such as differences in vulnerability toward dangers). This means that an exact knowledge of all three sex ratios is required before one can analyze the underlying causes for the deviation from the normal ratio.

The primary sex ratio of wild birds is easily obtained because it equals the secondary sex ratio in all those broods where the complete clutch of eggs hatches. It is, therefore, rather surprising that so little evidence on primary sex ratios in birds has yet been published. It is much more difficult to arrive at definite figures for the primary sex ratio in the domestic hen which lays continuously, particularly where heavy mortality in the eggs is involved. Consequently no data on this have yet been published. The actual figures on the primary sex ratio in the genera *Cassidix* (see p. 172) and *Larus* (see p. 158) will be quoted below.

The secondary sex ratio is reasonably well known for a number of domesticated species (see also Crew, *op. cit.*, p. 546). The greatest amount of information concerns the domestic fowl. All the evidence is summarized by Byerley and Jull (1935) who found among 96,008 chicks, alive at hatching,  $49.17 \pm 0.11$  per cent. males, a figure which deviates sufficiently from the ideal 50:50 ratio to be of statistical significance. In the pigeon, on the other hand, Cole and Kirkpatrick (1915) found a secondary sex ratio of 105♂:100♀. Crew quotes some additional figures, but in none of the cases (except in hybrids) did the

secondary sex ratio vary more than 5 or 10 per cent. from the ideal 50 : 50 ratio.

Very little is known about secondary sex ratios in wild birds. There are, of course, scattered through the ornithological journals quite a number of statements concerning the number of males and females among the young in a single nest, but the number of nests that were investigated was too small to be of any statistical significance. The few available data indicate a rather high proportion of males among Passerine birds. All the evidence has been ably summarized by F. Groebels (1937).

How little such scattered data signify is particularly well illustrated by MacDowell and Lord's (1925) work on the house mouse, which showed an almost ideal primary sex ratio (416♂:415♀) in 106 litters. In single litters, however, the sex ratio was often unequal, for example: 3♂:6♀, 1♂:7♀, 7♂:3♀, 7♂:1♀, etc. Something very similar was shown by F. Goethe (1937) in his study on the primary and secondary sex ratios in the herring gull (*Larus argentatus* (L.)). In 14 complete clutches with 3 eggs he found the following sex ratios: 0♂, 3♀ (4 times), 1♂, 2♀ (3 times), 2♂, 1♀ (3 times), 3♂, 0♀ (4 times). In 11 clutches with 2 eggs he found once 0♂, 2♀, twice 2♂, 0♀, and 8 times 1♂, 1♀. The total of 64 young birds yielded 33♂ and 31♀, a remarkably close figure in view of the small number of clutches that were examined. This strong random variation invalidates the significance of most of the material quoted by the older ornithologists (Liebe, 1894; Heinroth, 1912; Lucanus, 1917).

Additional material on the secondary sex ratio in the genera *Accipiter* (p. 168), *Psaltriparus* (p. 166) and *Cassidix* (p. 172) will be discussed in later paragraphs.

#### THE TERTIARY SEX RATIO

Cytologists and geneticists are chiefly interested in the primary and secondary sex ratio, as illustrating certain cytological and genetic phenomena. The student of animal populations, the ecologist, the student of reproductive

behavior and the naturalist are, however, principally interested in the tertiary sex ratio, that is, the proportion of the sexes in adult populations during the period of reproduction. The great importance of the adult sex ratio has long been recognized by the student of human populations, but it has not received as much attention from the animal biologist as it deserves. The principal reason for this neglect has been the difficulty of determining the true sex ratio in adult populations. Furthermore, an "adult" population is never homogeneous, but rather composed of a whole set of age classes. There is also rarely a random distribution of the sexes because a number of environmental factors tend to segregate them. However, it can be shown that all these objections do not obliterate the value of the tertiary sex ratio, provided that a reliable technique was employed in the determination of this ratio.

#### METHODS AND SOURCES OF ERROR

The methods used for the determination of the tertiary sex ratio are largely determining the value of such figures. They can be used by the general biologist only if all possible errors are eliminated. A truly representative adult sex ratio can only be expected if the two sexes never segregate during seasonal movements nor differ in their habits to such an extent as to make counts unreliable. Such ideal conditions rarely exist in birds. How much these factors may influence sex ratios that are actually nearly balanced shall be shown in a few examples.

*The influence of migration:* Every ornithologist knows that the migration period of the male bird is in most species different from that of the female. The males usually return in the spring several days to weeks earlier than the females and may leave later in the fall. There is usually a preponderance of males in the winter populations of those species in which only part of the population is migratory, such as in the song sparrow (*Melospiza melodia*). M. M. Nice (1937) found during the breeding season an almost balanced sex ratio of this species in the



Interpont area near Columbus, Ohio. But owing to the departure of a greater proportion of the females, there were the following percentages of males caught in her traps (among more than 1,000 captures) during the winter months: September 52 per cent., October and November 70 per cent., December to February 74 per cent., March 67 per cent., and April–July about normal again. This stronger migratory urge of the females also expresses itself in the figures quoted by Drost (1935) for birds caught on the island of Helgoland during migration. In the eleven years 1924–1934, 1,092 males of the blackbird (*Turdus merula*) were caught during the fall migration against 1,631 females (59.9 per cent. ♀). During the spring migration the ratio was still more unequal, 1,846 males against 3,636 females (66.3 per cent. ♀). Still there is no evidence of a strongly uneven sex ratio during the breeding season. One of the best illustrations of the influence of migration time and situation of the winter quarters on the sex ratio is given in the figures published by Heydweiller (1936) on the composition of the flocks of tree sparrows (*Spizella arborea*) observed near Ithaca, New York. The proportion of sexes in these flocks was as follows:

October–December 15 .....	♂ 50–60%	♀ 40–50 %
January 1–March 15 .....	♂ 85	♀ 15
March 20–March 25 .....	♂ 70	♀ 30
March 28–April 16 .....	♂ 40	♀ 60
April 24–April 26 .....	♂ 15	♀ 85
May–September .....	♂ absent	♀ absent

A wealth of similar data can be found in the ornithological journals, particularly in *Bird-Banding* and *Vogelzug*. I have quoted all these figures merely to show that the enormous amount of similar evidence which has been published by the bird-banders is, unfortunately, useless for our purpose.

*Sexual dimorphism*: Another source of error in a study of the tertiary sex ratio in birds lies in their sexual dimorphism. The males of most species of birds are very much more conspicuous than the females. They are often more brightly colored, they sing and display, and, in the

case of territorial species, they are apt to inspect or even attack intruders. It is, therefore, not at all surprising that males predominate in random collections of birds. The great body of the figures compiled by Pelseneer (1926) is based on such collections and consequently useless for our purposes. The best proof for the fallacy of this method is given by the fact that museum collections always contain more adult males of the weaver birds (*Euplectes hordeacea* and *franciscana*) than females, although it is well known that in these species the females outnumber the males at least 2:1. Even in the species in which male and female are similar in coloration there is often a decided difference in behavior which may lead to error.

#### SEX RATIO AND LIFE HISTORY

Many ornithologists have failed to understand that the sex ratios in different families and even genera and species of birds are frequently quite different. For this reason it is wrong and illogical to say: The males outnumber the females among birds, or *vice versa*. Sound conclusions can only be reached if each group of birds is studied separately. A particular attraction is given to the study of the *tertiary sex ratio* in birds, because it is frequently correlated with some unusual mating behavior. It is, therefore, advisable to say a few words about pair formation in birds and to classify the occurrence of unusual sex ratios according to the pairing behavior in such species.

Birds are, as a rule, monogamous, at least for the duration of one brood. Occasionally, however, polygamy occurs, that is, several females settle in the domain or territory of one male and mate with him. In contradistinction we find polyandry in some other genera in which one female has several "husbands" who may take over a good share of the duties of incubation and raising of the young. Finally, we have a number of cases where no tie is formed by the sex partners, who separate completely after fertilization has been accomplished. This might be called "non-pairing."

## SURPLUS OF MALES

*Monogamous species:* Before we mention any of the species with very unequal sex ratios we might examine a monogamous species in which the number of males approximate that of the females. Fortunately there is available a very excellent and reliable study on such a species. M. M. Nice's (1937) study of a population of the song sparrow (*Melospiza melodia*) on a forty-acre tract at Interpont near Columbus, Ohio, has the advantage that the author had individually marked nearly every bird in this area and knew its entire history. It seems unlikely that the accuracy of this study of a wild bird population will soon be surpassed and it is therefore worth while to quote some of her figures (*l. c.*, Table XXIV):

ADULT SONG SPARROWS PRESENT AT INTERPONT AT THE BEGINNING (APRIL 6) AND DURING THE LATTER PART (JUNE) OF THE BREEDING SEASON

	April 6		June		Loss in per cent. of April population	
	♂	♀	♂	♀	♂	♀
1930 .....	30	30	27	25	10.0	16.7
1931 .....	41	41	36	34	12.2	17.1
1932 .....	69	65	61	51	11.6	21.5
1933 .....	44	41	38	31	13.6	24.4
1934 .....	29	25	23	16	20.7	36.0
1935 .....	25	25	20	16	20.0	36.0
Total for 6 years.....	238	227	205	173	13.9	23.8
First 3 years .....	140	136	124	110	11.4	19.1
Last 3 years .....	98	91	81	63	17.4	30.8

The first 3 years may be considered as representing normal conditions. During the last 3 years the habitat was disturbed and partly destroyed, resulting in a reduction of the population and an increased exposure to enemies. The consequence is higher mortality, particularly of the females, which in this species are more vulnerable to predation.

THE FLUCTUATION OF THE SEX RATIO (♂ PER 100 ♀)

	Males in April	Males in June
1930 .....	100	108
1931 .....	100	106
1932 .....	106	120
1933 .....	107	123
1934 .....	116	144
1935 .....	100	125
Average for 6 years.....	105	118
First 3 years .....	103	113
Last 3 years .....	108	129

Summarizing these figures, we can say that the sex ratio of this song sparrow population was nearly balanced at the beginning of the breeding season, particularly during the three years when the habitat was undisturbed. Heavy losses to the incubating females, however, caused a decided rise of the ratio, to a maximum of 144 in June, 1934. It is probable that the heavier losses of the territory proclaiming males in the spring account for the approximate balancing of the sex ratio in every subsequent spring.

It is the opinion of most field ornithologists that among many song birds the males outnumber the females by about 10-20 per cent. To prove such a contention involves so much work that it is rarely undertaken. It requires not only that the territory of each male be determined but also that each female and nest, respectively, be located. Only few species or genera lend themselves to such a study. In the European genus *Phylloscopus* (wood warblers) it has been proved by several authors for at least three species. H. v. Treuenfels (1937) found in a small wood in northern Germany that twelve of thirty-five males of *Phylloscopus sibilatrix* did not find a female. This indicates a sex ratio of 1.52 ♂ per female. Price (1935) found that in two populations of chiff-chaffs (*Ph. collybita*) of 63 and 42 males, 25 and 14 remained unmated (1.66 ♂ or 1.50 ♂ per female). In the same two tracts of woodland the numbers of male willow warblers (*Ph. trochilus*) were 90 and 53, of which 18 and 23 remained unmated (1.25 ♂ or 1.77 ♂ per female). Similar figures have been reported by other authors for the chaffinch (*Fringilla*), warblers (*Sylvia*, *Acrocephalus*), robins (*Rubecula*) and other species. Since there is no evidence in these species of unmated females, it is reasonable to conclude that these figures actually represent the proportions of sexes in these populations.

Hicks (1934) found among 2,522 individuals of the starling (*Sturnus vulgaris*) trapped in their winter quarters in Ohio that 1,714, or 67.9 per cent., were males. The sex ratio changed as follows from month to month:

Beginning of winter .....	December	234 ♂ : 100 ♀
Midwinter .....	{ January	180 ♂ : 100 ♀
	{ February	171 ♂ : 100 ♀
End of winter .....	March	230 ♂ : 100 ♀

The fact that the sex ratio stayed high throughout the winter (notwithstanding some variation) is one of Hicks's principal reasons for believing in the actual existence of an unequal sex ratio in this species. Kluijver (1935) finds a preponderance of males also in Dutch starlings, even in breeding populations.

In the American bob-white quail (*Colinus virginianus*) exact records are available in several eastern and southern states (Stoddard, 1932). Among 19,423 birds shot in Georgia and Florida there were 53.3 per cent. males (114 ♂ : 100 ♀). Among 10,707 quails shot at ten localities of five states over a period of 28 years there were 53.0–58.0 per cent. males. Many other observations, some of them based on equally large figures, support the contention of a ratio of 112–130 males to 100 females. Unfortunately, there are no data available of the sex ratio of freshly hatched young, but some figures indicate a higher post-natal mortality and consequently shorter average life of the female.

Some of the most remarkable cases of an unequal sex ratio seem to occur in the family of the honey-eaters (Meliphagidae). The earliest reference to this condition seems to have been made by Stresemann (1928) in regard to the genus *Myzomela*. This genus, comprising about a score of the species, is restricted to the Australian region and reaches its highest diversity on New Guinea. Most species are strongly dimorphic, with the males decidedly more vividly colored, but males and females usually feed together in little parties in flowering trees. The males have a rather inconspicuous song.

Ordinarily I would hesitate to pay much attention to the proportion of sexes among specimens in museum collections, but in this genus the evidence is so uniform and so overwhelming that I want to record it in detail. To make the case more convincing, it may be mentioned that

it has been fully substantiated by my own observations during two years of collecting in New Guinea and the Solomon Islands and also by the experiences of other observers. L. Macmillan, who has been studying and collecting the birds of the New Hebrides for years and has given special attention to the matter of sex ratio, writes me on November 16, 1937: "The females of *Lichmera* are outnumbered by the males at least 10:1, just as in *Myzomela*. T. H. Harrison and myself found this so on Malekula and I found it so on Erromanga and also here in Mare (Loyalty Islands)."

The Sepik Expedition collected the following number of males and females of the various species (Stresemann, 1923):

<i>Myzomela eques</i> : 30 ♂, 5 ♀ .....	85.7 per cent. ♂
<i>Myzomela cruentata</i> : 28 ♂, 2 ♀ .....	93.3 " " ♂
<i>Myzomela nigrita</i> : 16 ♂, 13 ♀ .....	55.2 " " ♂
<i>Myzomela rosenbergi</i> : 16 ♂, 6 ♀ .....	72.7 " " ♂

The richest material, with which to check the sex ratio in *Myzomela*, was probably collected by the Whitney South Sea Expedition.

*Myzomela jugularis* (from 42 islands, covering all the months of the year except March, April and May)—Fiji Islands

78 ♂ ad., 35 ♂ imm., 18 ♀ ad., 6 ♀ imm.—82.5 per cent. ♂

*Myzomela cardinalis chermesina*—Rotumah Island

16 ♂ ad., 12 ♂ imm., 8 ♀ ad., 7 ♀ imm.—65.1 per cent. ♂

*Myzomela cardinalis nigriventris*—Samoa

60 ♂ ad., 3 ♂ imm., 7 ♀ ad., 6 ♀ imm.—82.9 per cent. ♂

*Myzomela cardinalis tenuis*—New Hebrides

73 ♂ ad., 34 ♂ imm., 24 ♀ ad., and imm.—81.7 per cent. ♂

*Myzomela cardinalis sanctaecrucis*—Santa Cruz Islands

58 ♂ ad., 5 ♂ imm., 14 ♀—81.8 per cent. ♂

*Myzomela cardinalis sanfordi*—Rennell Islands

18 ♂ ad., 2 ♂ imm., 5 ♀—80.0 per cent. ♂

*Myzomela cardinalis pulcherrima*—San Cristobal

10 ♂ ad., 1 ♂ imm., 5 ♀ ad.—68.8 per cent. ♂

*Myzomela cardinalis malattae*—Malaita, Solomon Islands

34 ♂ ad., 13 ♂ imm., 12 ♀—79.7 per cent. ♂

(For further details about these species, see Mayr, 1932.)

I may add the percentage of males of some other species of the genus for which adequate material is available: *Myzomela lafargei*: 72 per cent.; *M. melanocephala*: 70.7

per cent.; *M. eichhorni*: 69.6 per cent.; *M. nigrata tristrami*: 61.8 per cent.; *M. cardinalis rubrata*: 52.8 per cent.; *M. cardinalis saffordi*: 75 per cent.; *M. cardinalis major*: 71.4 per cent.; *M. cardinalis dichromata*: 51.1 per cent.; *M. cardinalis kobayashi*: 70.35 per cent. This list could be greatly enlarged.

It is difficult to interpret these figures. The easiest explanation would be to say that the collector picked out the brightly colored and conspicuous males and neglected the collecting of females. There are two objections to this explanation. The first is that there are many other genera with equally or even more pronounced sexual dimorphism, but no such lopsided sex ratios are found in collections of these genera. The second objection is that in many cases there are in the collection more dull-colored immature males (which are in some of the species even more plainly colored than the females) than adult and immature females combined.

It is nevertheless possible that a correlation exists between sexual dimorphism and sex ratio, since there seems to be the smallest surplus of males in the species with the least developed sexual dimorphism. There is nothing known of the life history of these birds which would help to explain this sex ratio. They seem to be monogamous. Unfortunately, they are tropical birds, with only two young, and it will be difficult to gather sufficient data on the secondary sex ratio.

The only other genus of this family in which the males outnumber the females (by about 8 or 9:1) is *Lichmera* (formerly *Stigmatops*). Remarkable in this case is the fact that in most of the species of this genus there is practically no sexual dimorphism, except for size.

A. Skutch reports (1935) of the Central American bush-tit (*Psaltiriparus melanotis*) that the males outnumber the females about 4 to 6:1, which leads to the interesting habit that the unmated bachelor males help the mated pairs to feed the young. At three nests there were 1, 1 and 3 extra males sharing the duty of feeding the young. Skutch

reports that the twelve young which fledged from the three nests were all males. The sex was, however, not determined by dissection and it is possible that the young female resembles the male rather than the adult female.

Following these typically monogamous species, I shall treat some others of which the sexual relationship might best be classified as monogamy, although they are not quite typical. (See below, p. 172, for the cowbird.)

In the ducks (Anatidae) a preponderance of males has been recorded by a number of authors. McIlhenny (1937b) quotes the following sex ratios (among others) of birds trapped in their winter quarters at Avery Island, La.:

Ring-necked duck ( <i>Nyroca collaris</i> )	961 ♂, 286 ♀ (335 ♂ : 100 ♀)
American pintail ( <i>Dafila a. tzitzihoa</i> )	2780 ♂, 1413 ♀ (197 ♂ : 100 ♀)
Blue-winged teal ( <i>Querquedula discors</i> )	1318 ♂, 752 ♀ (175 ♂ : 100 ♀)

Similar figures have been quoted in Lincoln (1932) from the enormous material gathered by the U. S. Biological Survey.

Frieling (1934) analyzes the field observations made in Germany of 21,764 ducks belonging to 10 different species and finds the following number of males per 100 females:

*Spatula clypeata*, 178; *Bucephala clangula*, 170; *Nyroca ferina*, 160; *Anas penelope*, 145; *Nyroca fuligula*, 137; *Anas platyrhynchos*, 130; *Anas querquedula*, 127; *Anas acuta*, 123; *Anas crecca*, 118; *Anas strepera*, 112.

These figures indicate less of a disproportion than those of the banders quoted above. A monthly analysis indicates that in some of the species the females prevail in certain months and differ from the males in migration time, routes and winter quarters. Considering all the evidence together the preponderance of males has been established beyond doubt.

The high sex ratio of ducks has been confirmed by an analysis of a breeding population (Furniss, 1935). It would be highly interesting to determine the secondary sex ratio of the domestic duck.

*Polyandrous species:* Polyandry, that is the mating of



several males with one female, occurs in birds only rarely. It is restricted to those species (except for the above-described occurrence of the pseudo-polyandry in one species of *Psaltriparus*) in which the female is the more active participant in the courtship ceremonies and the male assumes the duties of incubation. Even in these species there is not always a surplus of males, as has been pointed out for *Phalaropus* by Tinbergen (1936). In fact, in the case of the Wilson's phalarope (*Steganopus tricolor*) Wetmore (1926) believes, on the contrary, in a surplus of females. Polyandry, of course, means that there is a surplus of males, but it is rather difficult to find reliable figures, since most polyandrous species live in the tropics.

The polyandrous button-quails (*Turnix*) live in the densest grass, and exact counts of males and females are impossible. Captivity observations indicate that one female may have as many as eight or nine males (Stresemann, 1928).

The painted snipe (*Rostratula*) is also known to be polyandrous. Pitman (1912) found in India in an isolated nesting colony 4 females with 12 males, and Stuart Baker (1935) found one female with 4 males at a different locality. This would suggest a proportion of 3-4♂:1♀, but more material must be gathered to substantiate these figures.

Beebe (1925) estimates that in the tinamou (*Crypturus variegatus*) there were about 4 males to every female. In the population in British Guiana which was studied by him, there were 32♂ and 8♀.

#### SURPLUS OF FEMALES

*Monogamous species:* A surplus of females in normally monogamous species has been recorded only from one order of birds, that of the hawks (*Accipiters*). Among 291 goshawks that were killed on migration there were 110♂ and 181♀ (Wood, 1938). This might be explained by the greater migratory urge of the females, but it is supported by a few data on the secondary sex ratio in

the European sparrowhawk (*A. nisus*). Gunn (1912) examined two nests in England and found in one nest 1♂, 5♀, in the other 2♂, 4♀. Maniquet (1927) examined three nests in France and found only four males among the fifteen young. The total sex ratio of the five broods is 7♂ and 20♀, or about 3 females per male. There is, however, also some conflicting evidence, particularly in other genera of hawks, and it is advisable to gather more material before one speculates on the possible reason for this surplus of females (see also Mayr (1938)).

*Polygamous species:* More and more evidence has accumulated in recent years to show that polygyny is not as rare in birds as formerly believed. It occasionally occurs as an exceptional condition even in species in which there is normally a surplus of males as in the song sparrow (Nice, 1937). But in addition to these exceptional cases, which have been reported from a number of families of birds, polygyny is a normal condition in certain species of at least seven different families of birds. The bond between the male and his females is sometimes very weak and it would probably be more correct to classify such species as "non-pairing."

Only one case of polygyny is known in the heron family (Ardeidae). Zimmermann (1929) showed that one male of the European bittern (*Botaurus stellaris*) may have several females and this observation has been confirmed by several other ornithologists (J. Vincent, U. von Sanden). It is of course impossible to ascertain the exact sex ratio in this secretive marsh bird.

The majority of the gallinaceous birds seem to have a fairly even sex ratio. The common fowl, which is polygamous in its domesticated state, is a descendant of the jungle fowl, which according to some observers (but not according to others) is monogamous. It is a general statement in the ornithological literature that the peafowl (*Pavo cristatus*) is polygamous. Several observers state that they have seen males accompanied by little harems of 5 to 6 hens. These field observations do not harmonize

with the experiences of a well-known breeder of this species, who writes me: "I can tell you absolutely that over a period of many years in breeding peafowl in confinement, the numbers of males and females are just about equal. . . . This covers and is true of both the Blue and the Green birds, as well as the varieties produced in domestication: Black-winged, White and Pied" (C. L. Sibley, *in litt.*). There is some evidence to suggest a surplus of females in certain genera of the Tetraonidae, as for example in the black cock (*Lyrurus tetrix*). In the big flight of the sharp-tailed grouse (*Pedioecetes phasianellus*) in 1932 there were 48♀ and 17♂♂ in 65 killed specimens. But nothing is known about the sex ratio on the breeding grounds (Snyder, 1935).

In the family of the bustards (Otidae) there is also some evidence for a surplus of females. I have, however, been unable to find any reliable counts.

The females also greatly outnumber the males in many genera of hummingbirds (Trochilidae). Nicholson (1931) writes of the Guiana king hummingbird (*Topaza p. pella*): "The dullish green females and immature males would be seen a hundred times or more for each glimpse of the old male." The apparent sex ratio in this population was 8♂:30♀, although various factors made it difficult to ascertain whether or not this was numerically correct. The fact that immature males resemble the females and that the males do not share the duties of incubation and raising of the young makes exact counts impossible. On the other hand, each male has its definite territory and lookout posts which helps to determine their actual number in a given region.

These are the only cases among Nonpasseres that have come to my attention. Among the songbirds polygyny occurs more frequently. Its occurrence is, again, restricted to certain families. In the family of the buntings (Emberizidae) most species are strictly monogamous. The exceptions that occur in the song sparrow have been mentioned above. Some cases of polygamy have also been

reported for the white-crowned sparrow (*Zonotrichia l. nuttalli*) (Blanchard, 1936), but the only species in which polygyny occurs regularly is the corn bunting (*Emberiza (Miliaria) calandra*). Ryves (1934) found in a first rough survey, in 1932, 27 nests in the territory of 16 males, which indicates a sex ratio of 100♂:169♀. In a somewhat more thorough investigation in 1933 he found 45 nests with females in the territory of 24 males, indicating a sex ratio of 100♂:187.5♀. Although the males are very conspicuous, the females are shy and retiring and it seemed probable that some of them had been overlooked. Ryves therefore contented himself in 1934 with a smaller area but covered it very thoroughly. In this area there were 15♂:51♀. This means a sex ratio of 100♂:340♀. All the 1934 males were polygamous, four had two females each, seven had three females, two had four females and two had seven females each. There is no reason to doubt these figures, since each male and each female was held under observation for a considerable period.

The family of birds in which polygyny seems to occur most often is that of the weaver birds (Ploceidae), and this has been known for many years.

W. W. Bowen (1926) observed in the Sudan two small colonies of the bishop bird (*Pyromelana franciscana*) which are sufficiently isolated to make his observations reliable. One of these colonies consisted of five females with a single male, which jealously guarded his territory against the intrusions of other males. In the other colony the single male had apparently a dozen females. Probably the most exact observations of any such species are those of David Lack (1935) on the crimson-crowned bishop bird (*Euplectes hordeacea hordeacea*) of East Africa. Of five males that were held under observation for several weeks, one had one female, two had two females, one had three, and one three or four. Polygyny for the same species and also for *Euplectes nigroventris* (1♂:3-4♀) is also reported by Vaughan (1930). The observations of Ali (1931) indicate a surplus of females in the Indian weaver

bird (*Ploceus philippinus*). No actual figures are given, but a male usually has two females, sometimes three, and it seems that the males are outnumbered by the females about 2:1.

The most interesting family for a study of sex ratios is probably that of the Icteridae (American blackbirds) because it contains some genera with an equal sex ratio, some with a surplus of males and some with a surplus of females. An equal sex ratio occurs probably in the majority of the species; there is evidence available to support it in the case of the bronzed grackle (*Quiscalus aeneus*) (Snyder, 1937), the Brewer's blackbird (*Euphagus cyanocephalus*) (Linsdale, 1938) and the Baltimore oriole (*Icterus galbula*) (own observations).

A surplus of males occurs only rarely in this family. It has been claimed both for the shiny cowbird (*Molothrus bonariensis*) and the common cowbird (*M. ater*) (Friedmann, 1929). Two or three males per female are said to exist, but only positive evidence seems to be banding record of 1,902♂ and 769♀ (2.47♂ per ♀) (McIlhenny, 1937b). There is considerable doubt about the sex ratio of the red-winged blackbird (*Agelaius phoeniceus*). Cases of polygamy in this species have been reported by several authors (Allen, 1914, Linsdale, 1938), but among trapped birds males outnumbered females about 3 to 1 (McIlhenny, 1937b) and more males than females are also found to occur among the young in the nest (Herman, 1938).

Polygyny is firmly established in three species of Icteridae. Chapman (1928) found, in 1926, in an isolated colony of Wagler's oropendola (*Zarhynchus wagleri*) on Barro Colorado, Panama, six or seven males and 39 females and in 1927 five or six males and 29 females indicating about 5 ♀ per male. Polygyny has also been observed in the yellow-headed blackbird (*Xanthocephalus xanthocephalus*) (Linsdale, 1938, Ammann, unpublished) with about 2 females per male. In the boat-tailed grackle (*Cassidix mexicanus*) not only the tertiary sex ratio has been studied but also the secondary. McIlhenny (1937a)

found a great preponderance of females in several colonies of this species at Avery Island, Louisiana. It is rather difficult to obtain reliable figures on the sex ratio of this species, which is not pair-forming, but among 874 feeding birds that were trapped and banded in 1935 there were 271♂ and 603♀, and among 974 birds trapped in 1936 there were 338♂ and 636♀, which corresponds to 31.0 per cent. or 34.7 per cent. ♂.

In 89 nests in which the young reached the age at which sex could be definitely determined, the following sex ratios prevailed:

Secondary sex ratio:				Primary sex ratio:			
1 nest	contained	2 ♂ only		3 nests	2 ♂ and	1 ♀	
8 nests	"	1 ♂ "		28 "	1 ♂ "	2 ♀	
3 "	"	2 ♂ and	1 ♀	6 "	0 ♂ "	3 ♀	
26 "	"	1 ♂ "	1 ♀	—	—	—	
28 "	"	1 ♂ "	2 ♀	37 "	34 ♂ "	77 ♀	
6 "	"		3 ♀ only				
15 "	"		2 ♀ "				
2 "	"		1 ♀ "				
—							
89 "	"	70 ♂ "	135 ♀				

The secondary sex ratio is thus 34.1 per cent. ♂ and 65.9 per cent. ♀, and the primary sex ratio 30.6 per cent. ♂ and 69.4 per cent. ♀, fluctuating approximately within the same limits as the tertiary sex ratio.

### DISCUSSION

Three questions present themselves to the student of the above-given evidence. First: What are the changes of sex ratio between the time of fertilization (primary sex ratio) and adulthood (tertiary sex ratio) and what factors cause them? Second: How can the existence of an unequal primary sex ratio be reconciled with our present knowledge of the genetics of sex determination in vertebrates? And third: What is the biological significance of uneven sex ratios?

To answer the first question, we must analyze all the known cases where (primary), secondary and tertiary sex ratios of one species are known. In the domestic pigeon

Cole and Kirkpatrick (1915) found a secondary sex ratio of 105♂:100♀ and recorded that there was, aside from some minor fluctuations, no significant change of the sex ratio with advancing age. In the domestic fowl the pre-natal (egg stage) mortality of the females is significantly, but very slightly, higher than in the males. A really high sex-linked lethality occurs, so far as I know, only in species and genus hybrids and in some closely inbred domestic varieties.

There is little published evidence on mortality in the eggs of wild birds. The few data which I have been able to gather indicate that completely sterile eggs occur not uncommonly, but that embryo mortality in the egg due to lethal factors and not due to chilling or other environmental factors is quite rare. However, no definite figures are available. As a matter of fact, even in the domestic fowl, there are only few sex-linked lethals known. There is every reason to believe that lethals are of no importance for the explanation of strongly unbalanced sex ratios in birds.

We know next to nothing about internal physiological factors that might influence sex ratios in birds. It is possible that the males have a greater longevity in certain species, on account of greater resistance to diseases and the rigors of the environment, but the available data do not permit sound conclusions. Spontaneous sex reversal (masculinization) has been observed in the domestic fowl in exceptional cases, but this phenomenon is unlikely to be of major importance. A complete determination of the secondary sex characters by sex hormones is, according to more recent research, probably rather an exception than the rule. Sexual dimorphism is apparently determined genetically in most species of birds.

Environmental factors are in many species of birds largely responsible for the difference between secondary and tertiary sex ratio, and there is no question that these factors are much more important than physiological factors; furthermore, they are more easily investigated.

They principally consist of a differential vulnerability of male and female to certain dangers. As has been stated above (p. 162), male song sparrows suffer the greatest losses during the spring period of territory establishment and courtship, while female song sparrows are most vulnerable during the period of incubation. A similar high mortality of females during the breeding season occurs probably in all the species in which the female carries the whole burden of incubation, particularly if the species is ground nesting. The above quoted lack of females among wood warblers (*Phylloscopus*) and ducks might possibly be explained on this basis.

Even if we take all these factors (lethal, physiological and environmental) into consideration, many cases remain in which there is little doubt about an actually unequal primary sex ratio (*Psaltriparus*, *Myzomela*, *Accipiter*, *Ploceidae* and *Icteridae*).

The only species in which actual figures are available (see p. 172) is the boat-tailed grackle (*Cassidix mexicanus*). McIlhenny (1937a) reports a primary sex ratio (only complete clutches counted!) of 30.6 per cent. ♂ and 69.4 per cent. ♀, a secondary sex ratio of 34.1 per cent. ♂ and 65.9 per cent. ♀, and tertiary sex ratios of 31.0 per cent. ♂ and 69.0 per cent. ♀ (1935), and 34.7 per cent. ♂ and 65.3 per cent. ♀ (1936). These figures show not only that the primary sex ratio in this species is very uneven, but they also indicate that no major shift in the sex ratio occurs during the lifetime. The variation of males between 30 and 35 per cent. is probably due to the small numbers.

The final conclusion is that in birds the primary sex ratio is frequently very unequal, just as Crew (1937) had maintained. This leads us to our second question: How can the existence of an unequal primary sex ratio be reconciled with our present knowledge of the genetics of sex determination in vertebrates?

Our knowledge of the chromosomes of birds has been computed recently by Miller (1938), who records that the sex inheritance follows the wo (♀), ww (♂) scheme in the



gallinaceous birds and in the Passeres, the only two orders of birds in which modern investigations are available. In other words, so far as we know, the males are homozygous and the females heterozygous for the sex factor, and we should expect a primary sex ratio of 50 per cent. ♂ and 50 per cent. ♀. The fact that so many exceptions to this ideal ratio occur indicates that some additional factors combine with *w* in the sex determination. Crew (1937) formulates this as follows: "... there should be genes which affect the functioning of the heterogametic mechanism." The differences in the sex ratios of different strains of canaries reported by Heape (1907) also suggest the presence of such modifying factors. Such genes have been described for several species of *Drosophila*. In the case of birds, they probably affect the oocytes in the ovary prior to ovulation. The occurrence of strong and weak sex factors (as in *Lymantria*) is less probable, since we would expect in this case intersexes which in birds are almost unknown.

Friedmann's (1927) attempt to correlate unequal sex ratios with the testicular asymmetry that is so common in birds does not seem very successful. Not only is testicular asymmetry known from many species with equal sex ratio, but furthermore the sex-determining mechanism is unlikely to exist in the males, if the female is the heterozygous sex. This, of course, has not yet been established beyond doubt, since, as stated above, the number of investigations is still small and restricted to a small percentage of the known families of birds. There is an urgent need for more cytological and genetic investigations on the sex determination in birds in order to gain a more secure foundation for an interpretation of the phenomena observed in nature. A full knowledge of the facts may force us to a more physiological interpretation of sex determination, and Riddle's work on these problems may appear in a new light.

Commenting on the significance of unequal sex ratios, Crew (1937: 554) comes to the conclusion that they are

correlated with sex-linked mortality and that the overproduction of one sex serves to establish a reserve against such mortality. It seems to me that this conclusion is much too restricted and that unequal primary sex ratios may also occur in connection with an increased demand for one sex during adulthood, in all species with special habits such as polygyny, polyandry, etc. Unquestionably sex determination in vertebrates is a good deal more complicated than is generally recognized.

The writer wishes to thank Professors L. C. Dunn, W. Landauer and A. H. Miller and Mrs. M. M. Nice for reading the manuscript and for many valuable suggestions.

#### SUMMARY

(1) The principal purpose of this paper is to show that in birds a great deal of well-documented evidence exists proving the occurrence of strongly uneven sex ratios.

(2) The sex ratio may be high or low, favoring the male or the female sex.

(3) The evidence points to the existence of this unbalanced condition already in the primary sex ratio, although physiological and environmental factors may modify it during prenatal and postnatal life.

(4) In nearly all well-studied cases unequal sex ratios have been found to be correlated with peculiarities in the life history of the birds.

(5) The study of the cytology and genetics of sex determination in birds is a promising and practically untouched field.

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SHORTER ARTICLES AND DISCUSSION  
CHROMOSOME ASSOCIATION IN *MESOSTOMA*  
*EHRENBERGII* (Focke) Schmidt

*Mesostoma ehrenbergii* of the order Rhabdocoela was reported from Europe by Focke in 1836 and first found in America by Woodworth in 1897. Graff (1913) states that it has been reported from numerous places in Europe and Asia, from Kansas, Michigan, Ohio, Illinois and New York in the United States, and from Trinidad. The chromosome number of the European *M. ehrenbergii* is five haploid and ten diploid according to Luther (1904), Bresslau (1904) and Voss (1914). The figures of meiotic anaphase I given by Voss, and Bresslau's drawings of the first cleavage show clearly that the European form has five pairs of chromosomes with approximately median centromeres.

During the fall of 1937 we obtained specimens of *M. ehrenbergii* collected from the Genesee River between the lower falls and Lake Ontario. This race appears to be structurally the same as the European form but different in chromosome number, chromosome morphology and probably in chromosome behavior. The animals from the Genesee River have the largest Turbellarian chromosomes reported up to this time. Each pair of chromosomes can be distinguished morphologically from the others. One of these pairs is never associated at meiotic metaphase. The eleven individuals which have been examined have the same chromosome number, morphology and peculiar meiotic behavior.

Eight chromosomes are found at mitotic metaphase (Fig. 1):

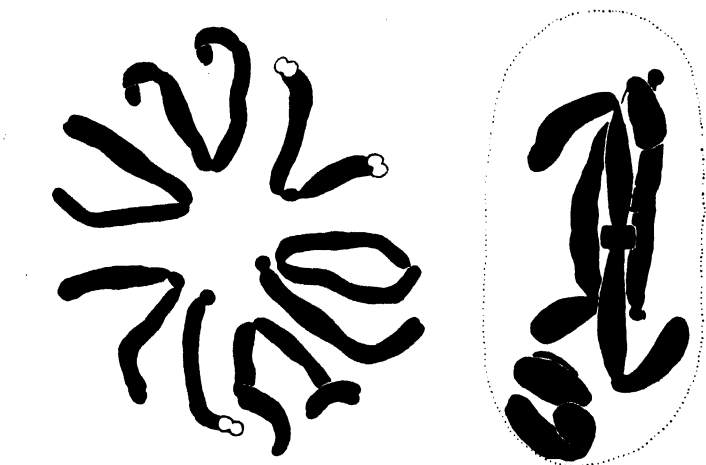
(1) Two chromosomes have subterminal centromeres and are approximately  $14\ \mu$  long.

(2) Two have nearly median centromeres and are approximately  $17\ \mu$  long.

(3) Two are from  $2-3\ \mu$  longer than those of pair 2 and have a second constriction about  $1\ \mu$  from the nearly median centric constriction. The two constrictions at metaphase are indistinguishable.

(4) Two chromosomes have nearly median centromeres and are approximately  $27\ \mu$  long.

The chromosomes are approximately  $1.2\ \mu$  in diameter and are usually distributed on the spindle with their long arms reaching into the cytoplasm. All the chromosomes show relic coiling, which persists in some of them until anaphase.



FIGS. 1 and 2. Chromosomes of *M. ehrenbergii*.  $\times 2500$ . 1. Mitotic metaphase. 2. First meiotic metaphase.

The meiotic prophase chromosomes are crowded in the small nuclei and with the methods employed so far are rarely fixed in a satisfactory manner. As the nuclear membrane of the primary spermatocyte disappears, the cell becomes elongated, presumably correlated with the formation of a long and narrow spindle. Three rod bivalents are usually oriented on the spindle at metaphase I and two univalents remain in the cytoplasm (Fig. 2). Although approximately one hundred cells have been studied at this stage in eleven individuals, no cell containing four bivalents has been seen. The two univalents are found at opposite ends of the cell, or in ca. 38 per cent. of the cells, at the same end. The uniformity of the situation and the presence of two univalents at the same end of the cell precludes the possibility that they result from precocious bivalent separation. The occurrence of univalents results in the formation of secondary spermatocytes which do not contain a uniform number of chromosomes. If these cells produce functional sperms, individuals which differ in the number of their chromosomes may be found.

The three bivalents can be readily identified. The members of the first pair of chromosomes which have subterminal centromeres are always associated. Either one, or in ca. 20 per cent. of the cells, two chiasmata are present in their long arms. When two occur, one is always terminal. The average chiasma frequency of this bivalent is 1.216.<sup>1</sup> One bivalent is attenuated near

<sup>1</sup> This and the data which follow are based on an analysis of sixty cells.

one or both centromeres in all the cells. A constriction, probably the centric constriction, is often seen in at least one of the free arms. This chromosome pair has an average chiasma frequency of 0.983. Approximately 70 per cent. of the chiasmata present are terminal. The arms of this pair are considerably shorter than the associated arms of the one previously described. Because of size and the presence of an attenuated region it is considered to be the third chromosome pair. The remaining bivalent is longer than the other two. It has an average chiasma frequency of 0.983. Over 30 per cent. of the chiasmata are terminal. It is undoubtedly the fourth pair of chromosomes.

Chromosomes "one" are always associated at metaphase I. Chromosomes "three" are found as univalents in one cell of the sixty selected at random, and chromosomes "four" as univalents in another. The first pair of chromosomes in one fifth of the cells has two chiasmata so only this proportion of cells has an average chiasma frequency of 4. Although four chiasmata are formed in a cell, the smallest bivalent contains two of them. If three are formed, the first chromosome pair, the attenuated bivalent and the long bivalent contain them (with the exception of one of the two cells which contain four univalents). In the one cell which contains only two chiasmata, the first pair of chromosomes and the third are the only chromosomes associated. It is clear that in the cells which contain three bivalents, the easily distinguishable first and fourth pairs are always associated. The third bivalent is always attenuated at one or two regions. If this attenuation is characteristic of a particular chromosome pair, *always the same chromosomes are unpaired in M. ehrenbergii at metaphase I.*

The attenuated bivalent has been considered to be the third pair of chromosomes because of its size and because the members of the third pair of chromosomes have a second constriction near their centromeres. The second constriction may be the region which becomes stretched into a slender thread at meiotic metaphase. It has been suggested that regions of chromosomes sometimes become drawn into thin threads by the stretching of the spindle. Either chromosome pair "two" or "three" because of its smaller size might be in the region of the spindle where the stretching action may be most effective. The members of the second pair of chromosomes have no second constriction near their centromeres and should be expected to behave like the first pair.

This pair, because it is often associated by chiasmata that are not terminal, is frequently oriented in the same general region of the spindle as the attenuated bivalent which usually has a terminal chiasma. Since the first pair of chromosomes has not been seen stretched into a thread, and an attenuated bivalent is always present in the cells which contain three pairs of chromosomes associated, there can be little doubt that the attenuated bivalent is always the third pair of chromosomes and that the members of the second pair are contracted less and never associated at meiotic metaphase.

Until a suitable technique is developed for the study of pachytene in *M. ehrenbergii*, we can not determine whether the situation here is due to asynapsis or failure of chiasma formation after normal pairing. Since the European form has ten diploid chromosomes and this form eight, the supposition that two chromosomes in the American *M. ehrenbergii* are without synaptic mates is an attractive one. It is not supported, however, by a study of the only other described species of *Mesostoma* in America. This species, *M. virginiana*, has eight chromosomes which regularly form four bivalents.

It can not be assumed that the unpaired chromosomes are paired at pachytene but are too short for the formation of chiasmata to occur because the shortest chromosome pair has the highest chiasma frequency. Arm length, however, may be a factor. The unpaired chromosomes are not the shortest in the complement, but they have the shortest arms. The third chromosomes appear to have slightly longer arms. Seventy-one per cent. of the chiasmata in this pair are terminal. The fourth chromosomes have arms of about equal length and approximately the same length as the long arm of the first chromosome. The fourth chromosomes have the same average number of chiasmata as the third pair, but only thirty-two per cent. of them are terminal. Its size may prevent sufficient association to assure a higher chiasma frequency.

It is probable that the arms of the second chromosomes are long enough for the formation of chiasmata to occur in the light of Mr. R. I. Bosman's unpublished work on the related flatworm *Gyratrix hermaphroditus*. This species has four diploid chromosomes. Two have subterminal centromeres and are rarely associated at meiotic metaphase. Two chromosomes have median centromeres. These measure approximately two times the length of the other pair and are associated by chiasmata which are local-



ized at the centromeres. When the chromosomes with subterminal centromeres are associated, chiasmata are usually but not always localized at the centromeres. Insufficient length alone can hardly explain the subterminal chromosomes being nearly always unpaired in *Gyratrix*.

The failure of chromosome association at meiotic metaphase has been shown to be the result of a recessive gene by Beadle (1930) and others and to environmental conditions by various workers. The effect in either case seems to be variable. The complete absence of association and nearly normal bivalent formation are often found in the same individual. In the eleven specimens of *M. ehrenbergii* we have seen, no cell containing all the chromosomes paired is present. The third pair of chromosomes is never associated. This peculiar situation to our knowledge has not been previously encountered.

A more detailed study of the chromosomes of the American species of *Mesostoma* will be reported elsewhere.

Since this article was accepted for publication, we have received a paper by A. Valkanov (Cytologische Untersuchungen über den Rhabdocoelen. Jahrbuch der Universität Sofia, Physico-Mathematische Fakultät, 34: 321-402, 1938) in which he describes the chromosome behavior in the European form of *M. ehrenbergii* and in the other species of Rhabdocoela. Valkanov's paper has not been translated but in his German summary he writes: "Während der Spermatogenese wird die parallele Konjugation noch im Pachitän gestört und durch eine späte Metasyndese, Amphimetasyndese oder durch einer völligen Isolation der homologen Chromosomen ersetzt. Bei *Mesostoma Ehrenbergii* ( $2n=10$ ) bilden sich auf dieser Weise im Prophase der Reductionsteilung 3 Tetraden und 4 univalente Chromosomen, bei *M. lingua* ( $2n=8$ ) bilden sich 4 Tetraden oder 3 Tetraden und 2 univalente Chromosomen, bei *M. sp. II* und *Rhyncomesostoma rostratum* bilden sich stets nur univalente Chromosomen."

The old world *M. ehrenbergii* which Valkanov has studied differs from the American *M. ehrenbergii* in chromosome morphology and number but the two forms do not seem unlike in the behavior of their chromosomes at the first meiotic division in testis tissue. Four chromosomes fail to associate in the form with ten chromosomes while two of the eight chromosomes are unpaired in our material.

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THE HINDRANCE TO GENE RECOMBINATION IMPOSED  
BY LINKAGE: AN ESTIMATE OF ITS TOTAL  
MAGNITUDE

STUDIES of hybridization in natural populations have shown (Riley, 1938; Delisle, 1937; Goodwin, 1937; Anderson and Hu- bricht, 1938; Anderson and Turrill, 1938) that the characters of the parental species tend to stay together in such populations. While this had been predicted (Anderson, 1936) from a consider- ation of the linkage of multiple factor characters with each other, the phenomenon is of such practical and theoretical importance as to deserve rigid mathematical exposition. The following paper attempts to estimate the hindrance to free recombination imposed upon hybrids by gene linkage alone. It does not consider the effect of such other restraints as pleiotropy, selective fertilization, zygotic elimination and gametic elimination.

It has been immediately apparent to those who have considered the question that linkage greatly reduces the chances of recombina- tion. Jones (1920), for instance, has computed the chances of recombining favorable genes in maize. "Two factors in each chromosome so spaced as to have 10 per cent. breaks in the linkage with each other would necessitate  $20^{20}$  individuals in the segre- gating generation to have an even chance of securing the one plant desired. This number of corn plants would require an area roughly 3,700,000,000,000 times the area of the United States."

The restraint of linkage, however, is not combined to frequencies. It also imposes severe restrictions upon the kinds of gene combina- tions which are possible with any frequency. When all the loci of a germ-plasm are considered this restriction is even greater than that imposed upon frequencies and runs into figures of astronom- ical magnitude. Some notion of this restriction may be gained by

considering recombination in a single cross-over segment of the germ-plasm. Let us take the simple case of a short chromosome in which there is regularly a single cross-over. Let us further suppose that in the two species, or races, which are to be crossed, there are ten pairs of gene differences within this chromosome. This seems a conservative number for a length of germ-plasm which might well be fifty units long genetically and made up of two hundred or more genes.

In the gametes of the first generation hybrid, as a result of four-strand crossing-over, one half of the gametes will have one crossed-over section in this chromosome and the other half will have none. The number of cross-overs per chromosome will be increased the same way in each generation; double cross-overs will not be possible until the  $F_2$  generation forms its gametes, triple cross-overs until the  $F_3$ , etc. In each generation one half the gametes will acquire an extra cross-over, one half will continue the previous number. The number of cross-overs per gamete and the proportions of each kind of gamete can therefore be obtained from expanding  $(\frac{1}{2} + \frac{1}{2})^n$  where "n" equals the number of hybrid generations. For the ten gene pairs under consideration complete recombination can not be attained until gametes are produced in which all nine breaks between the original sets of ten differing gene pairs have occurred. To obtain such a gamete will require a minimum of nine hybrid generations, and even then these gametes may be expected only once in  $2^9$  (=512). It will require twice as many hybrid generations before gametes of this degree of recombination will be in the majority. The above figures have all been obtained on the hypothesis of random crossing-over along the chromosomes. Actually, of course, cross-overs would tend to recur in particular areas and thus greatly reduce the possibility of complete recombination.

A more precise estimate of the hindrance to recombination can be obtained by considering the ratio of the possible gene combinations in the  $F_1$  to random combination. With three pairs of differing loci,  $\frac{abc}{ABC}$ , there can be a cross-over between the "a" locus and the "b" locus and between the "b" and the "c." Each of these will permit two recombinations, viz., aBC, Abc; and abC, ABc. The total number of recombinations will therefore be equal to twice the number of gene abutments or  $2(n-1)$  where "n" equals the number of differing gene pairs. With the two original com-

binations the total number of kinds of gametes will be  $2n$ . Since the total number of possible combinations, were it not for the restrictions imposed by linkage, is given by  $2^n$ , the ratio we are seeking will be  $2n/2^n$ . For three pairs of gene differences this becomes  $3/4$ ; for four pairs  $\frac{1}{2}$ ; for ten pairs  $10/512$ , or less than 2 per cent.

Since the same principle will be operating in every cross-over region (tempered only by the occurrence of multiple crossing-over) the total hindrance in the entire germ-plasm will be enormous. An estimate can be obtained by considering the not impossible case of an organism which regularly has a single chiasma in each chromosome. For such an organism the ratio of the possible kinds of gametes to the total number of recombinations will be  $\left(\frac{2n}{2^n}\right)^N$

where " $n$ " equals the numbers of differing loci per chromosome and " $N$ " is the number of pairs of chromosomes. For even such a slight difference as four genes per chromosome and with only six pairs of chromosomes this ratio becomes  $1/64$ . For ten gene differences per chromosome and with ten pairs of chromosomes it becomes  $(10/512)^{10}$  or roughly less than one in 100,000,000,000,000.

It should be emphasized that this restriction is independent of the size of the  $F_2$  and constitutes an absolute *upper* limit to gene recombination in that generation. The ratio  $(10/512)^{10}$ , inconceivably small though it may be, represents the fraction of the total combinations which could be achieved in a population of infinite size. In any actual  $F_2$  the additional restrictions of combination frequencies will reduce the actual gene combinations to a fraction of this infinitesimal fraction.

It may therefore be predicted that linkage alone will greatly hinder recombination in species crosses, or in any cross where there is a considerable number of genes involved. Even with small numbers of gene differences in each cross-over segment, the possible recombinations among the gametes of the  $F_1$  will be only a fraction of the total imaginable combinations. With any considerable number of gene differences the possible combinations will be only an infinitesimal fraction of the total combinations, and numerous hybrid generations will be required before there can be anything like complete recombination of those gene differences which have survived. These theoretical considerations suggest that the con-

ditions in the hybrid populations which have been studied are general phenomena.

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#### CHANGE IN GASTRIC DIGESTION OF KINGFISHERS WITH DEVELOPMENT

DURING our studies of the belted kingfisher (*Megaceryle alcyon*) in its relation to salmon (*S. salar*) and trout (*Salvelinus fontinalis*) we have had opportunities of making some observations on the digestion of fish by this bird.

The kingfisher has no crop. The proventriculus is not developed to the extent that it is in many birds. The muscles of the gizzard are thin and apparently unfitted for performing any vigorous grinding action. The gizzard, being thin walled and distensible, is not unlike the stomach of carnivorous mammals. The intestine has a small lumen and is long and symmetrically coiled above the gizzard (intestine of young kingfisher 6.8 times length of bird).

#### DIGESTION IN NESTLING

We have reared several young kingfishers and have examined the stomach contents of nestlings which had been preserved in formalin. The young will consume daily from one to one and three quarter times their weight in fish. Before the development of the flight feathers, *i.e.*, until they are about a month old, the young birds will digest all the bones and scales of the ingested

fishes (even when fed artificially on bony pieces of large adult fishes). While making food analyses of nestlings which had been collected from the nests, we were able to verify the observations made on the reared birds. The stomachs showed bones in various stages of digestion, and in some instances all the fish bones had been dissolved with some of the flesh still undigested. There was some softening of the chitinous parts of insects, but considerable quantities, from the food of digested fishes, had accumulated in their stomachs. When washing the stomach contents of the nestlings, even when there was little trace of other fish remains, there was generally considerable free fish oil floating on the water.

#### DIGESTION IN FULLY FLEDGED BIRDS

A short time before the complete development of the flight feathers the young, as found during rearing, began to disgorge stomach pellets of partly digested fish bones. By the time the young are sufficiently fledged to leave the nest, digestion of bones has ceased and thereafter they disgorge the complete skeletons, even of very young fishes, as well as the larger scales of the fishes which they have eaten. Large numbers of bone pellets may be found along streams beneath kingfishers' perches. We have used these pellets in our studies of the food of the kingfisher. The bones are well cleaned without breaking, there is no erosion or absorption and they make excellent material for the identification of the species of fishes captured by the birds. The larger scales are equally well cleaned and we have used them for age determination of the fishes. The chitinous parts of the insects from the stomachs of ingested fishes are disgorged with the bones.

For the purpose of food analysis many fully fledged kingfishers (adults and juveniles) were shot and preserved in formalin. When these were examined it was found that the condition of the stomach contents confirmed the observations made on the pellets. The food showed no evidence of any grinding and in many of the stomachs, pellets were found in various stages of formation. When washing the contents there was seldom even a trace of free fish oil on the water.

Observations on the reared birds showed that during their development they disgorged no pellets until near the time when they became fully fledged. The examination of the stomach contents of nestlings and fully fledged birds has shown in the former all stages in the dissolution of bones and scales, a slow softening

of chitin and no saponification of fish oil, whereas the latter have shown no digestion of bones, fish scales or chitin but a rapid saponification of fish oil.

These observations indicate a change from an acid reaction in the stomachs of the nestlings to an alkaline reaction in the stomachs of the fully fledged birds.

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PRELIMINARY EXPERIMENTS ON THE COLOR  
CHANGES OF *ANOLIS CAROLINENSIS*  
(CUVIER)<sup>1</sup>

No one can observe the American chameleon, *Anolis carolinensis* (Cuvier), in a garden, yard or field without being impressed by the readiness with which it changes in color from brown to green and *vice versa*. Because its life is largely spent in an environment of green and brown the phenomenon of metachrosis is attributed to the function of protective coloration. However, under continued observation the chameleons are frequently seen to be green on a brown background or brown on a green background. These observations suggest the possibility that the American chameleon reacts to the quality as well as to the intensity of light. Light perception, either qualitative or quantitative, to bring about bodily changes in color must be through photoreceptors (the eyes, parietal eye or receptors in the skin) or by direct action of light rays on the chromatophores in the skin. It is well known that in darkness these lizards turn green. No references mention a reaction to any of the colors of the spectrum.

To clarify the sometimes confusing observations on the color changes in the field, chameleons were collected during April and May, 1938, and the following experiments carried out under laboratory conditions.

- (1) Brown and green individuals were placed in darkness.

*Result:* All animals became green.

- (2) Brown individuals were placed in a glass jar covered with green Cellophane and exposed to light, either daylight or artificial light.

*Result:* Chameleons turned green.

- (3) A green hood of Cellophane was fastened over both eyes and the parietal eye; and these specimens were then exposed to daylight or artificial light.

<sup>1</sup> The writer is deeply indebted to Dr. L. H. Kleinholz, of Harvard University, for encouragement and for criticisms of the manuscript.

*Result:* Turned green or stayed green as long as the green hoods were kept in position.

- (4) Green hoods were placed over both eyes and a black patch was fastened over the parietal eye and the individuals were exposed to daylight or artificial light.

*Result:* The same as in 3.

- (5) Black patch over parietal eye only and the individuals exposed to daylight or artificial light.

*Result:* Same color reactions as shown by the specimens with no eyes covered.

- (6) A black hood was placed over both eyes and the individuals exposed to light, either daylight or artificial.

*Result:* All specimens turned a deep chestnut brown in sharp contrast to other chameleons in the cage whose eyes were uncovered and which were yellowish brown in color.

- (7) A black hood was placed over both eyes and the parietal eye and these specimens exposed to daylight or artificial light.

*Result:* Same as in 6.

- (8) A black patch was fastened over one eye and a green hood over the other eye and these specimens then exposed to daylight or artificial light.

*Result:* Same color reactions as when both eyes were covered with green Cellophane.

- (9) Chameleons as described in 6 and 7 were placed in a glass jar, covered with green Cellophane and exposed to daylight or artificial light.

*Result:* Stay deep chestnut brown in color.

## DISCUSSION

These experiments indicate that the parietal eye plays no part in light perception for color changes. Parker (1938) reached a similar conclusion in his study of *Phrynosoma*.

The light passing through the green Cellophane and then into the eyes brings about the green phase. This result was duplicated many times. Lizards with the green hoods were kept in a wire cage for as long as four days with unhooded chameleons as controls: the unhooded animals were consistently yellow-brown during the daytime. Individuals with the black hoods over their eyes turned a deep chestnut brown when kept in the same wire cage with the green-hooded chameleons and their controls. The differences between the three groups were striking and conspicuous. During the experiments one individual fitted with a green hood turned a deep chestnut brown when placed in the cage. Examination showed that the green hood had been applied so tightly that the eyelids of this animal were held in the closed position. When the green hood was loosened and the chameleon again placed in the



cage, it turned green and stayed green as long as the hood was kept on. A few chameleons with green hoods or in glass jars covered with green Cellophane appeared yellowish-brown instead of the expected green. When they were watched carefully, it was noticed that they were rather sluggish and were alternately closing and opening their eyes for short intervals. They were thus between the green phase to be expected as the result of the green hoods and the deep chestnut brown phase expected of those individuals whose eyelids were closed for long periods of time with the body exposed to light.

The above experiments check with the writer's observations of chameleons in the field. In conditions where green leaves serve as a heavy green screen over the chameleons, they are green, regardless of background. The writer has watched green chameleons climb from under dense green foliage so that they were exposed directly to the sky light. These individuals then turned brown and were very conspicuous against the green background.

#### SUMMARY

Light, even after passing through green Cellophane, acts on the body alone (not through the eyes) causing a dark brown chestnut phase (extreme expansion of the chromatophores).

Light passing through a green Cellophane filter into the eyes inhibits the expansion of the chromatophores or causes their contraction so that the chameleon is green. Intermediate shades are the result of differing proportions of green light coming into the eyes, determining the extent of chromatophore inhibition.

Absence of light entering the eyes and the absence of light on the body also inhibits the expansion or causes the contraction of the chromatophores.

Green light can stimulate the retinal receptors to effect the green coloration but is not an effective stimulus for dermal photoreceptors or the chromatophores which can not perceive changes in the quality of the stimulus.

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## COLOR CHANGES IN ANIMALS, THEIR SIGNIFICANCE AND ACTIVATION<sup>1</sup>

### INTRODUCTORY REMARKS

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ANIMALS are marvelously resourceful in meeting the impact of their surroundings. Even such inert and apparently innocuous creatures as sea-anemones and jelly-fishes can often inflict a sting excruciatingly painful to man and deadly to many fishes. Thus these simple inhabitants of the sea protect themselves against insult and kill other creatures for their food. All animals generate electricity in greater or smaller amounts. To the expert physician the beating of the human heart can be read and studied in terms of almost imperceptible electric changes, but the electric eel of South America can deliver a bolt that will knock down a horse or kill a frog. All animals secrete substances which may vary from the hardness of the needle-like fang of a rattlesnake to the fluid venom injected through such a fang. Heat is produced by all animals and light by a goodly number. The luminous creatures of sea and land have been the inspiration of poets from the days of Homer to the present time, and Oriental nations celebrate as national festivals the coming of the firefly. Bodily movements and their attendant locomotion are commonly looked upon as animal responses par excellence. Most such activities are accomplished by

<sup>1</sup> A symposium given at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.

muscles which give us the lumbering gait of an elephant, the astounding flight of a bird, the beat of an insect's wing so rapid often as to produce an audible note and the unequalled modulations of the human voice. But animal movements are not only muscular. They may be due to the whipping of innumerable, minute, protoplasmic lashes or cilia which like myriads of microscopic oars propel small water creatures through their aqueous environment. Or these movements may be due to the sluggish flowing or snail-like creeping of the living substance of such microscopic animals as the ameba whose semi-fluid body seems to flow over the solid background of its watery abode. Nor are we in our own bodies devoid of such activities, for our white blood-corpuscles migrate within us by the same means that the ameba uses and assemble where they are needed to attack and destroy such invading organisms as the germs of disease.

To this form of motility, a kind of viscous or sarcoous streaming, are to be attributed many of the color changes in animals. These changes are commonly carried out by the displacement of the colored contents within certain cells, the chromatophores, located in the skins of those animals that easily change their tints. When myriads of such cells of a given shade and in the integument of a chromatic animal disperse their pigment, they confer on the creature their characteristic tone. When these cells draw in their special coloring matter and concentrate it about their centers, the tint previously shown by the animal disappears. Thus the creature by controlling the movements of its various chromatophoral pigments may change its color over a range that is limited only by the number of kinds of color cells in its skin. Any one who has watched a flounder swimming over a diversified, sandy bottom will have observed the play of colors that pass over its body as cloud shadows pass over a landscape. So regular and progressive are these waves of color as they course over the skin of this fish that it seems almost incredible that they should be due to an orderly repeated

expansion and contraction of a multitude of color cells. Yet such is the truth.

That animals are able to change their tints was known to the ancients, for Aristotle, the father of natural history, described these phenomena in the chameleon and in the devil-fish. His account of these changes is so circumstantial and detailed that we are forced to believe that he must have been acquainted with them at first hand. The means by which such changes are brought about were discovered, however, only a little over a century ago and the term chromatophore as the name for the integumentary agent concerned with such changes was first used in 1819 by the Italian Sangiovanni. Shortly after that time it was discovered that the eyes of chromatic animals were essential to their color changes, for when these organs were effectually covered or the animal otherwise blinded, it ceased to respond to color differences in its surroundings. The involvement of the eyes in color responses led to the assumption that the color cells of animals were under the control of their nerves and this view, well supported by much experimental evidence, maintained itself throughout the last century. Since the eyes and other nervous parts of animals were thus included in their chromatic organization, it followed that this type of system was to be expected only in the higher creatures. It was therefore not surprising to find that color changes with very few exceptions proved to be limited to these animals. The ability to exhibit color changes is found among certain mollusks such as the squids and devil-fishes, among crustaceans as represented by the shrimps, prawns and crabs, and, lastly, among the cold-blooded vertebrates, the fishes, amphibians and lizards. All these animals are highly organized in that they possess well-developed eyes and nervous systems capable of high degrees of efficiency. Nor was there before the end of the last century reason to suppose that more than these parts were necessary for a full understanding of animal color changes.

For many years past it has been known that the injec-

tion of certain drugs and other like materials into the bodies of chromatic animals is often followed by changes of color. In 1898 it was discovered by two Italians that when small amounts of adrenalin were injected into frogs, these animals became very pale in tint. Adrenalin is a product of the adrenal gland, an organ of internal secretion, and its function is that of an activator or hormone, to use a technical designation. Thus an internal secretion in the form of the hormone adrenalin was shown to be a possible means by which color cells could be brought into action. Less than two decades after this, intermedin, the hormone from the intermediate lobe of the pituitary gland of the brain, was seen to have a most profound influence on the chromatophores of many animals. The effect of this hormone was the reverse of that of adrenalin, for while adrenalin made animals pale, intermedin made them dark. As a result of these discoveries and of others of a like kind, it was shown that color cells in animals were controlled not only by nerves but by internal secretions in the form of hormones or, as they are specifically termed nowadays, neurohumors.

But this distinction, important and significant as it seemed to be, is apparently doomed to disappear, for the most recent work on this subject indicates that the nervous control of color cells is in reality accomplished also by secreted substances, substances that are liberated from the ends of the nerves as other activating materials are discharged from glands. If this view proves to be correct, then all instances of chromatophoral activation, nervous as well as hormonal, are dependent upon these substances, some of which, like intermedin, come from distant glands and are carried to the color cells by the blood and others from nerve endings in closest proximity to the reacting color elements themselves. Such is the most recent opinion of the way in which color changes are controlled. By a delicate adjustment of the amount of these activating substances or neurohumors, by a balance of such agents, so to speak, and by their appropriate interplay, any chro-

matic animal may attain to a given tint or succession of tints such as is needed in its daily life.

The conception that the cells of the nervous system and of its appended parts, such as muscles, glands and chromatophores, are linked together not by electric intercommunication as believed of old but by activating substances such as these described in this brief survey is steadily gaining ground. It is the working hypothesis of a host of modern investigators in nerve physiology. That human thought with all its implications, both normal and pathological, as well as so distant an activity as the color changes of animals should rest upon a background of chemical activation is a view that promises rich and important yields in the future investigation of the whole range of nervous phenomena.

It must be evident from what has been said in these introductory remarks that the subject of color changes in animals is one of fascinating complexity. Its investigation may be carried out from many standpoints. The papers which make up this symposium will lead you to see how diversified this attack may be.

# CHROMATOPHORES IN RELATION TO GENETIC AND SPECIFIC DISTINCTIONS<sup>1</sup>

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WE as zoologists are familiar with the extraordinary diversity of the characteristics of living things. Our classifications are based largely on structural similarities and diversities. The lesser subdivisions, such as genus, species, variety and Mendelian phenotypes, usually utilize the more readily visible characters. Among the latter are colors and color patterns—as, for example, the distinction between the zebra and the horse. My purpose in this paper is to survey the relative degree to which these taxonomic and genetic differences may be displayed by color patterns, by color cells—the chromatophores, and by pigments. Among these, however, I wish to pay especial attention to the cells. We may inquire as to what extent do chromatophores of different families, genera, species or genetic phenotypes exhibit recognizable distinctions. In accordance with prevailing beliefs we may state that the characteristics of the organism are conditioned by the interaction of all its genes; and moreover most of the cells of the individual are thought to contain this full complement of genes. May we then not expect to find that homologous cells in species A and B or even in genetic phenotypes A and B differ recognizably on account of their differing gene content? That this is to some extent true has

<sup>1</sup> Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938. When presented it was illustrated in part by lantern slides of color photomicrographs (Kodachrome) which can not be reproduced here. I am greatly indebted (especially for the photography) to my assistant, Miss Marian Hedenburg. Many of the photographs were taken at the New York Aquarium by authorization of the acting director, Dr. C. M. Breder, Jr., and with the help of Dr. C. W. Coates and Dr. R. F. Nigrelli, to all of whom I wish to express my thanks. Expenses incurred were defrayed by grant from the Denison Fund for Biological Research at Wesleyan University.

long been recognized by the comparative histologists and by the embryologists who take advantage of recognizable cell differences for demarking the boundaries of interspecific tissue transplants. A not infrequent statement made by students who work with individual living cells, either in the organisms or in tissue cultures, is that they can by long familiarity recognize the cells of different species, even though it is difficult to formulate these distinctions in words. Protozoologists distinguish many different species of *Amoeba* by criteria hard to describe, as they must be applied to a "formless" animal. It is my desire to call attention to a few observations that have been made bearing on the question of the possibly recognizable specificity of pigment cells of fishes.

We may first consider the phenotypic distinctions due to single gene differences. Perhaps the most notable and clear-cut cases are those described by Gordon in a series of papers dealing with *Platypoecilus maculatus* (Gordon, 1931). The character "spotted" is produced by very easily recognizable cells, the macromelanophores, and these appear only when gene "Sp" is inherited. Similarly, character "stippling" is caused by an abundance of micromelanophores which follow gene "St." When gene "Sp" is united in an interspecific hybridization with the genetic complex of the swordtail fish, *Xiphophorus helleri*, there occurs an extraordinary overdevelopment of macromelanophores forming distinctive melanotic tumors.

In *Oryzias* (*Haplocheilus*) *latipes*, first studied by Aida (1921) and later in greater histological detail by Goodrich (1927), a normally pigmented melanophore follows gene B, while the recessive allelomorph b produces a faintly pigmented cell. The difference between these two has been shown (Goodrich, 1933) to be apparently due to lack of sufficient chromogen in the light-colored cell, although sufficient oxydase is present. In this fish, however, the situation is complicated by the fact that a third allelomorph B' produces a situation such that approximately equal numbers of dark and light cells are produced. This



probably indicates that the control of these cell differences is at least in part due to factors in embryonic development other than the gene content of individual cells, such for example as an embryonic induction.

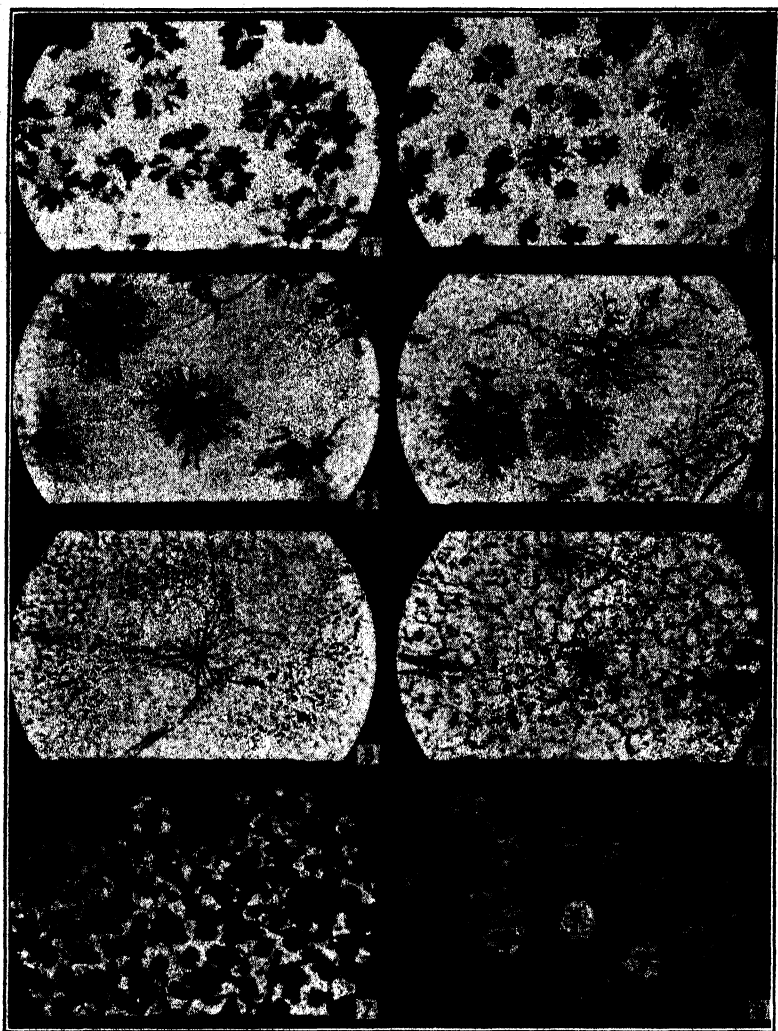
A very interesting interspecific cross between the mackerel, *Scomber scombrus*, and the killifish, *Fundulus heteroclitus*, was first made by H. H. Newman in 1918. A more detailed study of the history of chromatophores in this cross which confirms and adds to his observations is now being made by Dr. Alice M. Russell, who has kindly given me permission to refer to her unpublished work. The embryos resulting from this hybridization do not proceed to maturity. In embryonic stages, however, some individuals show, apparently unchanged, the distinctive green chromatophores of the mackerel and others the red brown chromatophores of the killifish, or both may be present in the same embryo. Here then the results indicate that the chromatophores follow the shufflings of the hereditary genic complexes. In this same cross, according to Dr. Russell's observations, it is more difficult to be certain of the relations of melanophores of the two parental types.

Single gene differences may control the presence or absence of cell types. This is illustrated by the total absence of melanophores in an albino mutant contrasted with the normal, as in the paradise fish, *Macropodus opercularis*, described by Goodrich and Smith (1937). There are many types of fancy tropical fish which probably only await genetic analysis to reveal cases of cell types controlled by single gene differences.

A second phase of our inquiry is concerned with the question as to how extended a distribution of practically identical cell types exists among related varieties, species or genera. This is a field in which at present there is insufficient material for forming the basis of a confident generalization. A preliminary exploration, however, indicates different degrees of constancy of cell types in different families and genera of fish. Gordon's studies on *Platyopocilus*, which revealed the existence of two types

of melanophores, as noted above, are now being extended (Gordon, 1937) in an investigation of the closely related *Xiphophorus helleri*, a fish which has an overlapping geographic range with *Platypoecilus maculatus* and which hybridizes with it. Here he finds the same type of micromelanophores as in *Platypoecilus* (Fig. 3, *Platypoecilus*; Fig. 4, *Xiphophorus*) and certain similar color patterns. On the other hand, as mentioned above the macromelanophores can not be introduced into *Xiphophorus* without disastrous results. Our observations also indicate that a distinctive erythrophore having red granular periphery and yellowish center is found in both of these related species. The genetics of a beautiful xanthophore which we have observed in a variety of *Xiphophorus helleri* known to breeders as the blood-red swordtail is as yet unknown. Varieties of *Mollienisia sphenops*, known to fanciers as the "blue mollie" and the "liberty mollie," have strikingly similar melanophores. Taking now an example of distinct species in the same family, Figs. 1 and 2 show the similarity of the heavy melanophores of *Macropodus opercularis* and of *Betta splendens*, species of the family Osphronemidae. Two species of the family Haemulidae, or the grunts, recently examined at the New York Aquarium have similar delicate melanophores (Figs. 5, 6). I have recently been interested in the family Labridae, or the wrasses, which includes many brilliant coral reef fish. Species have been studied at the Tortugas laboratory in the Gulf of Mexico and at the laboratory of the University of Hawaii, and although these were so widely separated geographically they show some degree of resemblance of the melanophores and especially of the iridocytes (Figs. 7, 8).

The similarities may seem more real when they are compared with existing diversities of cell types. The family Cyprinidae, or the carps, is very large and wide-spread, and genera show great diversity as is illustrated by photographs of cells from *Rasbora trilineata* (Fig. 9) and *Brachydanio rerio* (Fig. 10). Types from widely sepa-

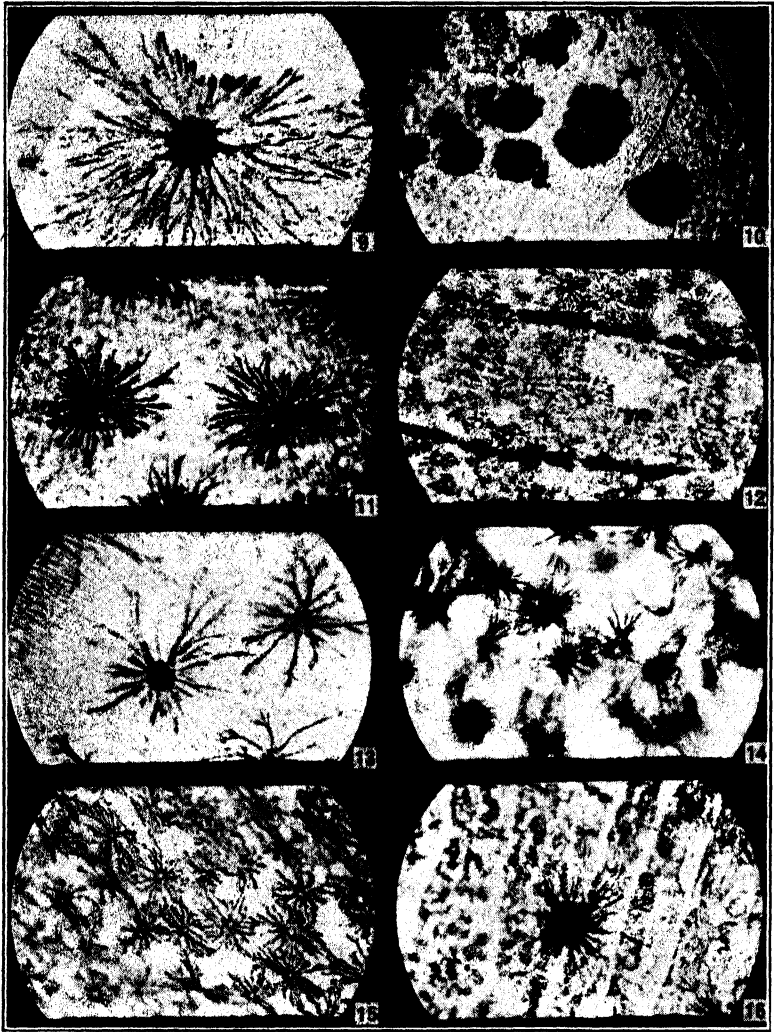


## PLATE I

Comparison of melanophores of closely related species or genera. Photomicrographs. Figs. 1-6,  $\times 163$ , Figs. 7-8,  $\times 88$  magnification. Family Osphronemidae. Fig. 1. *Macropodus opercularis* Linnaeus (Paradise fish). Fig. 2. *Betta splendens* Regan (Siamese fighting fish). Family Poeciliidae. Fig. 3. *Platypoecilus maculatus* Guenther ("Platy"). Fig. 4. *Xiphophorus helleri* Meek (swordtail). Family Pomadasidae. Fig. 5. *Haemulon macrostomum* Guenther (gray grunt). Fig. 6. *Haemulon flavolineatum* Desmarest (yellow grunt). Family Labridae. Photographs to show iridocytes, melanophores contracted. Fig. 7. Dark stripe on *Halichoeres bivittatus* Bloch (slippery dick). Fig. 8. Blue zone on *Thalassoma bifasciatum* Bloch (bluehead).

rated families are shown in Plate II. These include in addition to the carps the following: *Fundulus heteroclitus* (Fig. 11) from family Cyprinodontidae; *Lophopsetta maculata* (Fig. 12), family Pleuronectidae; *Holocentrus ascensionis* (Fig. 13), family Holocentridae; *Pseudoscarus guacamaia* (Fig. 14), family Scaridae; *Julis eydouxi* (Fig. 15), family Labridae; *Centrophistes striatus* (Fig. 16), family Serranidae.

The preceding discussion may have seemed to imply that I have held that the nature of cells is directly and wholly determined by their own gene content. But of course the possible influence of cell environment must not be forgotten. Indeed such an effect is clearly indicated by sex demorphism in such types as the "guppy," *Lebistes reticulatus*, and *Xiphophorus helleri*, and confirmed by cases of sex reversal and experiments with sex hormones (*cf.* Berkowitz, 1938). In this latter case there is the clear indication of the suppression of the male color pattern by feeding immature fish with the female sex hormone. These circumstances indicate that the differentiation of certain chromatophores, or even their presence or absence, may be dependent on chemicals circulating in their environment. The distinction between the control of a cell's structure directly by its own nuclear constitution and an effect produced on it by its environment is the same as that which has been drawn in recent literature in the field of physiological genetics and referred to as the difference between genic and endocrinal regulation of tissues, but what I would prefer to refer to as the distinction between immediate and remote genic control. Splendid examples of this are known from the studies on bird plumage (*cf.* reviews by Danforth, 1932, and by Domm, Juhn and Gustavson, 1932). For our immediate inquiry, however, we prefer to try to eliminate variables and so are concerned with cells which do not vary between the sexes or seem to be otherwise differentially affected by their environment (as in *Oryzias* cited above).



## PLATE II

Diverse types of melanophores. Photomicrographs all  $\times 163$ . Family Cyprinidae. Fig. 9. *Rasbora trilineata*. Fig. 10. *Brachydanio rerio* Hamilton-Buchanan (zebra fish). Practically no pseudopodia appear on these melanophores. Family Cyprinodontidae. Fig. 11. *Fundulus heteroclitus* Linnaeus. Family Pleuronectidae. Fig. 12. *Lophopsetta maculata* Mitchell (sundial). Family Holocentridae. Fig. 13. *Holocentrus ascensionis* Arbeck (squirrel fish). Family Scaridae. Fig. 14. *Pseudoscarus guacamaia* (rainbow parrot fish). Family Labridae. Fig. 15. *Juliseydouxi* Cuvier and Valenciennes. Family Serranidae. Fig. 16. *Centropristes striatus* Linnaeus (sea bass).

The photographs of chromatophores that we have shown indicate, I believe, that these cells exhibit in many cases a specific individuality, although the distinctions may be evasive and hard to analyze. Also in some cases chromatophores of related species and even of related genera show a considerable degree of similarity. The most obvious similarities are matters of the size of the cell and the form, length, branching and number of its pseudopodia. In all cases, however, caution is necessary, as the appearance of the individual cell varies greatly according to the degree of concentration or distribution of its contained pigment.

Turning now to the coloring material we may state that pigment appears to vary less frequently than the cell types. The structural formula of melanin is not yet known. It does not appear that there are many melanins. The coloring materials of xanthophores and erythrophores probably are carotenoids, and it has been suggested that there may be about 100 different carotenoids. Quite possibly the estimate may be increased by biochemists, but the order of magnitude is not likely to approach that of the approximate number of species of fish—about 15,000. Therefore we may assume that frequently the same pigment will appear in many different species of fish.

From the preceding discussion we may tentatively conclude that while a chemical cytoplasmic cell component, the pigment, may be wide-spread among many species, the cell type has a much more limited distribution, which may be less than or somewhat more extensive than the species. The most obvious and probably most real differences between species that are based on colors are caused by variations in the spatial arrangements of the color-producing units, *i.e.*, the cells. Effects are produced in two ways, by microscopic and by macroscopic groupings or patterns. The former may produce visually different colors. For example, brilliant blues are formed most frequently by the association of melanophores and iridocytes. Erythrophores or xanthophores may be present. In greens the

proportion of xanthophores is increased. No blue or green pigment is usually present. I have observed that the brilliant bluish color of the head region of a coral reef fish, *Thalassoma duperrey*, from Hawaii, is produced by a background of melanophores, iridocytes, and erythrophores upon which are superimposed little islands of melanophores and glittering iridocytes apparently floating on the transparent epidermis. The following table shows counts of chromatophores per square millimeter made from certain coral reef fish of the family Labridae.

TABLE I  
CHROMATOPHORES PER SQUARE MILLIMETER

	Stripes		
	Black	Blue	Green
<i>Thalassoma bifasciatum</i> (Tortugas)			
Melanophores .....	718	346	462
E plus X* .....	0-550	566	0-190
Iridocytes .....	3,000	3,000	3,000
<i>Thalassoma duperrey</i> (Hawaii)			
		Yellow	Green
Melanophores .....		271	596
Xanthophores .....		1,057	527
Iridocytes .....		3,000	3,300

\* Erythrophores plus Xanthophores.

The macroscopic effects are the obvious groupings which produce stripes, bands, spots, and in fact almost any conceivable configuration. The macroscopic groupings and possibly the microscopic arrangements are distinctive of each species. It is then in this realm of the larger units of organization that we find the maximum expression of the possibilities of biological color variation. The chemicals seem least variable, the cells more variable, and the configurations of color patterns most variable of all. This of course does not seem strange, because biologists, aided by the philosophers of science, have distinguished between various degrees of organization. We are familiar with the categories of electronic, atomic, molecular and biological levels of organization. And within the biological we distinguish between the cellular and the multicellular. The last, having available greater possi-

bilities of spatial arrangement, is logically the most variable and therefore the best level of organization for expression of biological specific differences.

Passing over this last philosophical digression, the chief points which I wish to emphasize are the following two: (1) that cells may have an individuality which like that of the whole organism is an expression of its genic complex, though probably more affected by some genes than by others, and (2) that the limits of distribution of a cell type may not always be coextensive with the species. Its distribution may be less or sometimes even greater than the species.<sup>2</sup>

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<sup>2</sup> Diagrams which illustrate excellently the idea that taxonomic characteristics may or may not vary with the species and quite independently of each other will be found in papers by Alfred C. Kinsey (Fig. 8) and G. G. Simpson (Fig. 7) presented in a recent symposium before the American Society of Naturalists.



# THE PITUITARY CONTROL OF CHROMATOPHORES IN THE DOGFISH\*

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THE color changes of elasmobranch fishes were not discovered until about six years ago. In spite of the numerous contributions during this short interval, there exists much diversity of opinion concerning the mechanisms controlling elasmobranch metachrosis. This paper will summarize the various present opinions of chromatic control, and in addition will present some original data which indicate the possibility of a basically uniform plan of chromatic behavior for this group.

The ability to adapt to white or black backgrounds is not shared by all elasmobranchs thus far investigated. These fishes may be roughly divided in this respect into two groups: one showing background responses (macroscopic or microscopic), which are evident within a few hours or a day or longer (*Mustelus canis*, Parker and Porter, 1934; *Raia branchiura*, *Raia maculata*, *Rhina squatina*, *Scyllium canicula* and *Scyllium catulus*, Hogben, 1936; *Squalus acanthias*, Waring, 1938; *Trigon pastinaca*, *Raia undulata*, Vilter, 1937) and another showing no background adaptation or, at best, limited responses visible only after many days or weeks of exposure (*Raia clavata*, *Raia batis*, Schaefer, 1921, *Torpedo marmorata*, Veil and May, 1938; *Urolophus testaceus*, *Trygonorrhena fasciata*, Griffiths, 1936). The first three species of the latter group are more or less persistently dark in tint. No diurnal rhythm of chromatic behavior has been reported in elasmobranchs.

When the pituitary gland or only the neuro-intermediate lobe of this gland is removed, all elasmobranchs thus far

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investigated become completely pale, due to extreme melanophore contraction, and remain in this condition in environments which elicit darkening in unoperated animals (*Mustelus canis*, Lundstrom and Bard, 1932; *Squalus acanthias*, Lewis and Butcher, 1936; *Scyllium canicula*, *Raia brachiura*, *Raia maculata*, *Rhina squatina*, *Scyllium catulus*, *Raia clavata*, *Raia microcelata*, Hogben, 1936; *Torpedo marmorata*, Veil and May, 1937; *Trigon pastinaca*, *Raia undulata*, Vilter, 1937; *Raja erinacea*, this paper). However, such hypophysectomized specimens may be darkened temporarily by the implantation of the neuro-intermediate lobe or by the injection of an appropriate extract from this lobe of the hypophysis. From this and other evidence to be pointed out later, all investigators are agreed that the dark phase of elasmobranch color change is due to the production and circulation of the chromatophorotropic hormone (intermedin, "B" hormone, melanophore hormone, etc.) of the neuro-intermediate lobe of the pituitary.

Although investigators are in complete agreement concerning the mechanism controlling the dark phase, no less than four theories have been proposed to account for the induction of the pale or light phase:

(1) That blanching or melanophore contraction is due to the production and circulation of a new hypophyseal hormone—the "W" or melanophore contracting hormone of the anterior lobe of the elasmobranch pituitary (the dualistic hormone theory, Hogben, 1936, Waring, 1938, pertinent to all elasmobranchs).

(2a) That paling is due to the direct intervention of melanophore contracting nerves, carrying visual impulses, initiated by a white background, to the melanophores by means of a lipoidal neurohumor as a chemical mediator ("The Nervous Theory," Parker, 1935; pertinent chiefly to *Mustelus canis* and also to *Squalus acanthias*).

(2b) That pallor is due to a tonic action of the nervous system allowed expression when the influence of the pituitary is removed or diminished. As in 2a, a neurohumor

(sympathin) is postulated as a chemical mediator for the induction of melanophore contraction. (Vilter, 1937; pertinent probably to all elasmobranchs).

(3) That pallor is brought about by the diminution or cessation of intermedin secretion during adaptive responses to a white background ("Unihormonal Theory," Lundstrom and Bard, 1932; Parker, 1936-1938; pertinent to *Mustelus canis*, *Raja erinacea*, *Squalus acanthias*).

The remainder of this paper will be devoted to an examination of these theories with respect to the original data obtained from experiments on the smooth dogfish, *Mustelus canis*. Such a procedure may be criticized on the general ground that various elasmobranchs differ in their mechanisms of control and, therefore, that a discussion of the group based on results of only one member is unwarranted. However, in *Mustelus*, evidence pertaining to all three main theories has been obtained, and consequently this species has proven to be for the present the most desirable experimental animal.

The responses<sup>1</sup> of *Mustelus* to backgrounds and to certain operative measures are shown in Table I. Although some of these responses have been described fully by Lundstrom and Bard (1932) and Parker (1936-1938), a quantitative tabulation is presented in this paper not merely for the purpose of verification, but chiefly to indicate the extent of variation. Three significant responses may be noted:

(1) That the neuro-intermediate lobe is the only portion of the pituitary, the removal of which results in complete melanophore contraction.

(2) That the removal of only the anterior lobe results in the loss of the white background response.

(3) That the melanophores do not respond directly to light. Concerning the third point, it is true that blinded elasmobranchs are darker when illuminated than when in

<sup>1</sup> The diameter of the pigment mass within the melanophores (melanophore diameters) was determined on living fish by means of a calibrated ocular micrometer. 10-25 melanophore diameters were recorded for each fish, and the average taken.

TABLE I  
MELANOPHORE RESPONSES IN THE PUPS OF *MUSTELUS CANIS*  
(After one day adaptation to background)

Condition of animals	White background			Black background			Darkness		
	Number of animals	M.D.	S.D.	Number of animals	M.D.	S.D.	Number of animals	M.D.	S.D.
Normal .....	5	74	16	10	191	18	4	141	3
Optic nerves cut .....	4	178	13	4	170	7	4	165	3
Complete hypophysectomy*									
plus Optic nerves cut ..	3	32	0.3	2	32	0.5	4	30	2
Complete hypophysectomy.	4	29.2	1.0	4	29.2	1.0	5	29.2	1
Neuro-intermediate lobectomy .....	6	32.4	7.0	5	29.2	3.0	5	27.5	2
Anterior lobectomy .....	4	186	13	5	196	8	2	181	12
Ventral lobectomy .....	4	90	8	6	144	24	2	152	32
Sacculus vasculosus extirpated .....	6	94	36	2	175	13	4	141	37
Neuro-intermediate lobectomy plus sacculus extirpated .....	2	29.2	0.3	1	37	...	1	32	..

M.D. = Melanophore diameter in micra.

S.D. = Standard deviation in micra.

\* = Extirpation of anterior, neuro-intermediate, and ventral lobes, and also sacculus vasculosus.

darkness. The justification of describing this response as a direct effect of light on melanophores (Hogben, 1936; Waring, 1938) is questionable, since it does not occur in blinded, hypophysectomized specimens.

We may now consider the first of the four proposed mechanisms for the induction of the pale phase—the “W” hormone theory.

#### (1) EXTIRPATION OF THE ANTERIOR LOBE

The removal of the anterior lobe results in the loss of the white background response, the animals remaining persistently dark in tone in spite of long sojourns on a white background. First demonstrated by Hogben (1936) for *Scyllium canicula*, *Scyllium catulus* and *Raja brachiura*, this effect has been confirmed by Waring (1938) for *Squalus acanthias*, and in this paper for *Mustelus canis* and *Raja erinacea*. That the loss of the white background response results specifically from the extirpation of the anterior lobe, thereby depriving the animals of a “W” hormone, is doubtful, as reference to Table II indicates. This table shows that the same response may be effected by

TABLE II  
EFFECT OF SUPERFICIAL BRAIN LESIONS MADE IN WHITE ADAPTED PUPS  
(*MUSTELUS CANIS*) ON MELANOPHORE RESPONSES  
(5 animals per group)

Type of lesion	Melanophore diameters in micra				
	On white back-ground	Placed on black on 4th day			Returned to white on 5th day
	1st	3rd	Days after lesion 4th	5th	6th
Transverse 1-2 mm between optic chiasma and anterior lobe .....	243	234	243	243	243
Transverse 1-2 mm anterior to optic chiasma .....	65	65	49	243	50
Transverse 3-5 mm caudad to pituitary .....	49	65	65	243	41
2 longitudinal lesions parallel with long axis of anterior lobe (3-5 mm) .....	40	73	97	243	110
"Denervated" pituitary <sup>1</sup> .....	240	240	240	240	240
"Implanted" pituitary <sup>2</sup> .....	240	240	240	...	...
Anterior and neuro-intermediate lobes separated, but not extirpated .....	162	180	200	180	190

<sup>1</sup> Tongue of anterior lobe dissected free from hypothalamus, then hypophysis completely freed from brain. Hypophysis attached now to brain case by ventral lobe.

<sup>2</sup> Same as above with the exception that anterior and neuro-intermediate lobes also cut free from ventral lobe.

superficial brain lesions made so as to interrupt the probable path of nerve supply (supraoptico-hypophyseal tract) to the intermediate lobe, even when the anterior lobe remains intact. In view of these experimental findings, therefore, the first line of direct evidence favoring the "W" hormone theory loses much of the significance generally attached to results following the surgical removal of an endocrine gland. The results of anterior lobectomy seem explicable as readily on the ground that the mechanism of inhibiting intermediate lobe secretion is interfered with as on the basis that a "W" hormone is no longer available to the melanophores. Certainly, in view of the results listed in Table II, the latter view can not be unequivocally maintained.<sup>2</sup>

## (2) REPLACEMENT THERAPY

If the anterior lobe produces a "W" hormone, implants of the anterior lobe or injections of extracts of it should

<sup>2</sup> It may be argued that brain lesions affect B and W hormone production differently. My experiments, of course, do not rule out the existence of a W hormone. They do show, however, that for the present the effect of anterior lobectomy must be regarded as non-specific.

restore the white background response in anterior lobectomized animals. Waring (1938) has found that such measures are wholly ineffective. By other tests (isolated dogfish skin, test on *Xenopus*) Waring (1936) and Hogben (1936) have not been able to demonstrate the presence, in the elasmobranch anterior lobe, of a substance which contracts melanophores.

### (3) TESTS FOR A "W" HORMONE IN THE BLOOD OF PALE ANIMALS

If a "W" hormone is responsible for pallor, its presence in the blood of pale animals should be detectable. Parker (1936-1938) injected blood of pale animals (*Raja erinacea*, *Squalus acanthias*, *Mustelus canis*) into dark specimens, without effect on the melanophores of the latter. My own attempts<sup>3</sup> to detect the "W" hormone in the blood of animals possessing intact anterior lobes but lacking neuro-intermediate lobes (such specimens should contain "W" hormone but no "neutralizing" B hormone) have also failed. In these experiments the test blood was injected intramuscularly into animals possessing intact intermediate lobes but lacking anterior lobes.<sup>4</sup>

### (4) DIFFERENTIAL TOLERANCE TO "B" HORMONE

Waring (1938) has shown that *Scyllium canicula*, when injected with a given dose of "B" hormone, will remain dark longer when the anterior lobe is absent than when it is present. This differential tolerance to equal doses of "B" is interpreted as being due to the presence of an antago-

<sup>3</sup> Subcutaneous injections of blood (0.2-0.5 cc) from normal pale, neuro-intermediate lobectomized and completely hypophysectomized animals into dark hosts (normal or anterior lobectomized) always produced pale spots. Sea-water likewise produced pale areas; hence the response is non-specific. None of these effects were produced on intra-muscular injections.

<sup>4</sup> It might be argued that these experiments do not rule out the presence of a "W" hormone in the blood since, by injection of pale blood into dark animals, any "W" present would be neutralized by the antagonistic "B" substance present in the host. Unless insufficient amounts of blood were injected, it is difficult to see how these experiments could otherwise be carried out.

nistic and neutralizing "W" hormone, hence animals with anterior lobes (and without neuro-intermediate lobes) do not remain dark as long as animals deprived of both lobes. Such a differential tolerance may be explained readily on other grounds: (1) that the anterior lobe may produce a substance inactivating "B" and yet not be a melanophore contracting hormone in itself, and (2), which is more likely, that the change in metabolism following anterior lobectomy in many animals may apply also to elasmobranchs, in which case the differential tolerance would be simply a differential metabolism of the injected "B" hormone.

Viewing all the evidence for the dualistic theory, it seems that a "W" hormone has not been demonstrated beyond reasonable doubt. The weakest evidence, peculiarly, results from the direct attack. The circumstantial evidence (differential tolerance curves) is stronger, but explicable on other grounds. The experiments herein reported do not rule out the existence of a "W" hormone. They do indicate, however, that the "W" hormone theory should not, for the present, be accepted until better direct evidence is obtained.

The nervous theory was first formulated by Parker (1935-1938), who discovered (Parker and Porter, 1934) that chisel cuts made transverse to the rays of the pectoral fin or that faradic stimulation of these rays produce pale areas. As a general mechanism of blanching in elasmobranchs, the nervous theory is not applicable, and Parker has limited it to *Mustelus* and possibly to *Squalus*, although not to all individuals of the latter species. Most of the elasmobranchs studied show no nervous control of melanophores. Section of spinal, trigeminal and facial nerves in *Scyllium* sp. has no effect on melanophores (Young, 1933). Similarly, negative effects have been obtained in *Scyllium canicula* by pectoral chisel cuts (Waring, 1936) or by section of spinal nerves (Wykes, 1936); in *Raja erinacea* by pectoral chisel cuts (Parker, 1937); in *Rhina squatina* and *Scyllium catulus* by electrical stimulation of spinal nerves and of the haemal canal (Wykes, 1936); in *Raia maculata*

by section of spinal nerves (Wykes, 1936); and in *Raia brachyura* by section of spinal nerves, or by faradic stimulation of spinal nerves or of the haemal canal (Wykes, 1936). In *Squalus acanthias*, electrical stimulation of several rays of the pectoral fin is ineffective in producing pallor (Parker, 1936) but chisel cuts across the rays sometimes produce pale areas (Parker, 1936; Waring, 1938). In *Torpedo*, Vilter (1937) induced pallor by faradic stimulation of the spinal cord. It appears therefore that more evidence has been accumulated for a nerve control in *Mustelus* than in any other elasmobranch, for in addition to cutting and stimulating experiments, Parker (1935) has been able to extract from pale fins a lipoidal neurohumor which he believes to be the chemical mediator to the melanophores.

It must be emphasized that a chisel cut in the pectoral fin of *Mustelus* does more than transect nerves. That the circulation distal to the cut may be interfered with is a criticism that has been repeatedly voiced. Parker has maintained, however, that collateral circulation is evident in the area affected by the cut, but whether this collateral supply is sufficiently normal in its rate of flow and in its total supply is as yet unknown. My studies of the arterial pattern of the pectoral fin indicate that in the area of the fin where cuts are commonly made, it is an anatomical impossibility to avoid severing several radial arteries in denervating by means of a chisel cut. If it could be shown that when nerves are surgically isolated and severed, the area thus denervated would pale, much of the doubt of the interpretation of pale fin bands would be dispelled.

By a dorsal incision over the pectoral girdle of *Mustelus*, the brachial plexus has been exposed and each nerve entering the plexus has been cut, but subsequent pallor in the fin has not been observed. That such an operation denervates the entire fin is seen from resulting immobility of the fin in swimming movements. Even when spinal nerves 1-13 were severed, no paling was observed. On the contrary, background responses may be induced in the denervated



fin. Similarly, section of the superficial ophthalmic nerve, on one side or both, produces no paling of the head region. Spinal nerves 21-26 were cut, either consecutively or alternately, but pale areas were not produced. If a chisel cut is made on a denervated pectoral fin, a pale band will appear within 10-30 minutes. If the fin of a dark fish is ligated, the entire fin will pale within 10-30 minutes. But, if a chisel cut is made at the same time the fin is ligated, no pale band will appear. Instead, the entire fin pales uniformly. When the ligature is removed, the fin will darken within 5-10 minutes, but the cut area will remain as a pale band. If a denervated fin is ligated, it will pale within 10-30 minutes.

From these results, it is evident that ligating a fin<sup>5</sup> and making a chisel cut produce the same response in exactly the same time. It is also evident that no melanophore contracting nerves are contained in the brachial plexus at the region of operation. Finally, it appears that if melanophore nerves exist in the pectoral fin of *Mustelus*, they must enter it at some point distal to the region at which the plexus enters the fin. In view of these results<sup>6</sup> showing both the importance of the blood supply and the non-existence of melanophore nerves in the brachial plexus at the point of operation, the nervous control of the pale phase in *Mustelus* can not be accepted wholly until the central connections of such melanophore contracting nerves are found or until histological examination reveals an innervation of the melanophores of this dogfish.

The thesis that melanophores are under a tonic nervous control meets with the same general objection—such nerves must be demonstrated first. An observation by

<sup>5</sup> It is possible that ligation of a fin stimulates the nerves by pressure, and hence the melanophores contract. The observation that section of the plexus close to the point where the ligature was made did not produce pallor can not be cited against such a possibility, inasmuch as the melanophore nerves could enter the fin without being contained in the spinal nerves, or if contained in the plexus, at some point distal to entrance of the plexus.

<sup>6</sup> The nerve-cutting experiments involving the brachial plexus were performed on pups, immature and mature specimens. Ligation experiments performed on pups.

Wykes (1936) that pallor is produced uniformly in innervated as in denervated areas following hypophysectomy tends to discredit the tonic theory. However, the argument that no melanophore nerves are present in the spinal nerves which were cut by Wykes is justifiable, as in the experiments cited above on severing the brachial plexus. But it must be reaffirmed that all attempts to demonstrate the presence of melanophore contracting nerves by surgical measures have failed, and that until this is successfully accomplished, the nervous theory can not be wholly accepted.

The unihormonal theory remains for discussion. Parker (1936-1938) found that defibrinated blood of dark but not pale animals produced melanophore expansion when injected into pale specimens of *Raja erinacea*, *Mustelus canis* and *Squalus acanthias*. Waring (1938) has also found that more "B" is present in the blood of *Squalus acanthias* during black adaptation than during white adaptation. My own results, although they are still in a preliminary state, are in accord.

Thus the unihormonal theory is the only one in which all the pertinent evidence is in favor of it, and against which there are at present no serious objections. Furthermore, it is the only plan of control which not only applies to those species for which it has been advocated, but which may also function as a basically uniform plan throughout the group. If concentrating nerves are present in *Mustelus*, it is conceivable that pallor is due to the combined action of both nerves and a diminution of intermediate lobe secretion, either of which could produce this effect alone. In any event, it is difficult to evaluate at present the relative significance of nerve control since, on the one hand, normal paling can be accounted for in terms of the pituitary alone, and, on the other, paling can not occur when the pituitary gland is incapacitated by denervation of other measures.

#### SUMMARY

Of the various theories of control for the color changes in elasmobranchs, the unihormonal theory is at present the

most satisfactory. This mechanism of control was intimated by Lundstrom and Bard (1932) the pioneer investigators in this field, and first clearly expressed and supported by Parker (1936-1938).

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# QUANTITATIVE EFFECTS OF VISUAL STIMULI UPON PIGMENTATION\*

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It is well known by this time, I trust, that the color changes of such animals as possess this capacity are caused in two quite distinct ways. On the one hand, we have relatively rapid changes, sometimes manifested within a few seconds' time, which involve no change in the amount of pigment, but merely the rearrangement of pigment already present. On the other hand, and less familiar, are the more gradual but more enduring changes, which result from the persistent action of adequate stimuli and which involve an actual increase or decrease in the amount of pigment in the animal's tissues. These types of color change have been distinguished, not altogether appropriately, as "physiological" and "morphological," respectively.

It is the former to which by far the greater amount of attention has been devoted in the past. It is the latter with which I shall occupy myself to-day. Owing to the limits of time I shall restrict myself to the pigmental changes of vertebrates.

We are not here concerned with the direct effects of light upon pigmentation, such as we have all experienced in the tanning of our own skins by the sunlight. The production or elimination of pigment to which I refer is brought about indirectly as a response to the background, and is mediated through the eyes and the nervous system.

So far as I know, the cytologist Flemming was the first to point out (1882) that the shade of the background could

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be influential in modifying the quantity of pigment in the skin of an animal. Flemming's observations were made upon larval salamanders.

But Babák (1912, 1913), using the same material, seems to have been the first to experiment with any thoroughness upon the effects of background on pigment formation, and to undertake quantitative comparisons of the resulting changes.<sup>1</sup> Babák counted several times, at intervals, the number of chromatophores in certain regions of the skin of living specimens which were kept on white and black backgrounds. He concludes: "There is thus no doubt that the nervous system, under the influence of the light relations of the environment, controls not only the movements and the mass of the pigment in the chromatophores, but also influences the division or multiplication of the chromatophores." Continued "expansion" of chromatophores, he believed, so altered the nutritive condition of these cells that there results not only an increase in their pigment but also a more rapid multiplication. Continued "contraction," on the other hand, produces reverse effects, though Babák's results indicated little, if any, loss of chromatophores which had once been formed.

Passing reference may be made to the claims of Kammerer (1913), who reported not only that young salamanders responded by pigmental changes to different backgrounds, but that these changes were inherited. Even the former of these contentions was disputed by Herbst (1919, 1924, 1927) at least for post-larval salamanders, though the experiments of von Frisch (1911) tended to support Kammerer's claims.<sup>2</sup>

Kuntz (1916) contributed a valuable paper on "The histological basis of adaptive shades and colors in the flounder *Paralichthys albiguttus*." He reports a reduction of approximately 30 per cent. in the number of melanophores in the superficial layers of the dermis when

<sup>1</sup> Franz (1910) made some brief observations of this sort on young plaice.

<sup>2</sup> I.e., as to effects on the first generation, but with no reference to inheritance.

specimens which had been kept for some time on white were compared with freshly caught specimens or ones from a black background. A graph which Kuntz presents shows both a decrease in fishes transferred to white backgrounds and an increase when such specimens were returned to black. The maximum effect, he found, was attained in 11 days. Moreover, complete adaptation to white probably involved an increase in the number of guanophores as well as a loss of melanophores.

The massive contribution of Murisier (1920-1921) must likewise be given prominence in any discussion of this subject. This investigator, working on young trout, found that white and black backgrounds strongly influenced the quantity of melanin produced, without appreciably influencing the growth of the fish. For one experiment he reports that the mean number of melanophores counted in the dorsal fins of the white-adapted fishes was 295, that for the black-adapted fishes being 680. Corresponding figures for the anal fins were 4 and 67. Murisier believed, however, that whereas sojourn on black leads to a great increase in the number of melanophores, sojourn on white merely causes an arrest of the further production of these cells, not a loss of those previously present. This is not in agreement with the findings of most others who have experimented on fishes.

Like Kuntz, Murisier was led to believe that stimuli which favored a decrease in melanin production favored an increase in the production of the white substance guanin.<sup>3</sup>

Blinding, he found, favored a great increase in melanin production, at least in fishes kept in the light. This has been the experience of several investigators,<sup>4</sup> including the present writer.

<sup>3</sup> Eva Meyer (1931) likewise contends that a continued sojourn on white leads to an actual increase in the number and size of iridocytes (guanophores). Sumner and Wells (1933) found evidence for an increase in the amount of guanin.

<sup>4</sup> Mayerhofer (1909) for pike, Babák (1912) and Fischel (1920) for Amphibia.

Of importance is the parallel which Murisier was one of the first to stress between the effect of retinal stimulation in bringing about the "contracted" condition of melanophores, and its effect in retarding the multiplication of these cells and upon the formation of pigment.

Himmer (1923) set out to test Babák's contention that morphological color change is a consequence of prolonged physiological color change. He performed the interesting experiment of subjecting larval salamanders to dilute solutions of cocaine, a substance which he found to produce a continued state of contraction of the melanin masses. Thus he aimed to eliminate the immediate effect of visual stimuli in the matter.

Himmer reports a resulting pallor, similar to, but smaller in degree, than that produced by life on a white background. Such a result plainly needs confirmation.

More recently, this same theoretical question has been discussed in the light of the hormonal interpretation of chromatophore action, which we owe so largely to the work of Parker and his collaborators.<sup>5</sup>

Giersberg (1934) attributes the melanin dispersion and subsequent melanin increase in blinded goldfish to the effect of intermedin from the pituitary. The expanded condition of the color cells, he believes, is the cause of the production of new pigment. "The morphological color change is hence to a certain degree a function of the physiological."

Giersberg reports that he injected a young brown goldfish for a period of a year and a half with adrenalin, and that the fish, in consequence, lost its yellow and black pigments. He suggests that life on a white background may likewise favor the production of adrenalin. We may remark even more emphatically in the present case that this experiment with adrenalin calls for confirmation.

Somewhat later, Odiorne (1937) has expressed the alternative view that physiological and morphological color changes are the parallel results of a common (hor-

<sup>5</sup> Cf. Parker, 1936. A later bibliography will be found in Parker, 1938.

monal) cause, rather than that the latter is a direct effect of the former.

To return to our chronological review, we must include certain other writers who have recognized the quantitative effect of visual and other nervous stimuli upon pigment formation.

Kurz (1920) exposed the eggs and larvae of *Pleuronectes* and *Esox* to white light, total darkness and to light of five different colors. No effects were observed in the case of the pike, after three months of exposure to these conditions. For the flounders, on the other hand, Kurz reported certain changes in the further development of pigment cells once produced, though not upon their origin.

The work of Kudô (1922) is sometimes seriously cited in discussions of this subject, but his entire report is so unconvincing, and his claims so incredible, that I can not attribute any importance to them. His thesis is that the darkening of a fish, however called forth and apparently of however brief duration, results from an actual increase in the amount of melanin present. Even "Tötung durch Schläge auf den Kopf" produces an increase in this substance! Pictures (*not photographs*) of test-tubes containing various amounts of dark precipitates are offered as evidence for these effects.

The findings of Vilter (1931) upon melanin production in axolotls should be mentioned at this point, though they deserve no such drastic criticism as do those of Kudô. They can hardly be regarded as convincing, however, owing to the small number of animals employed (one each on black and white) and to his very meager account of the methods employed.

Hewer (1927, 1931) working upon English flatfishes, found that an increase in epidermal melanophores occurred as a result of exposing his fishes for two or three weeks to a black background. The numbers both of erythrophores and iridiophores likewise were found to be influenced by the background, the former undergoing an



increase on orange and a decrease on white; the latter undergoing an increase on white.

I shall next report some of the results obtained by myself and associates during the past few years upon quantitative changes in the pigmentation of fishes.

In *Lebistes* (Sumner and Wells, 1933), there was a rather rapid loss of melanin when fishes of "black" history were transferred to a white background. After a few days, it required but low magnification to reveal the presence of numerous degenerating melanophores in the surface of the skin, while in sections large rounded masses of melanin were seen to be passing to the exterior through the epidermis. Returning such bleached fishes to black containers resulted, in a few days, in the appearance of numerous small pigment masses in the dermis, which increased in number and size for some weeks. These changes were not confined to the skin, however, for wide differences were found in the peritoneum and other regions between fishes of black and white history.

Transfers of the guppies back and forth between the two types of background showed that the same individual could alternately acquire and lose chromatophores. Photographs taken for the purpose did not reveal any tendency for newly formed pigment masses to occupy the same positions as those which had recently been eliminated.

Even the most prolonged exposure to a given background failed to render the resulting state of the chromatophores permanent. Fishes reared from birth in black or white containers, even when the light was continued night and day, and throughout their entire lives<sup>6</sup> responded fairly promptly to a reversal of backgrounds, though not so promptly as ones which had been kept for only a month upon a given background, after attainment of maturity.<sup>7</sup>

Our experiments were not quantitative, in the sense that

<sup>6</sup> Even some which represented a third generation reared under these conditions.

<sup>7</sup> These experiments relate chiefly to "physiological" color changes, but the same statements would doubtless apply to "morphological" ones.

an attempt was made at this time to count chromatophores or to measure melanin. But without any such determinations, the differences in question were sufficiently striking.

Odiorne (1933, 1936, 1937) experimented extensively in this field at about the same time that our La Jolla experiments were conducted. He employed fishes of a number of species, chiefly members of the genus *Fundulus*. Like several of his predecessors, Odiorne determined, in some cases, the extent of background influence in terms of the density of chromatophore distribution per unit area of skin. It is of interest that while the loss of melanin upon white backgrounds was everywhere manifest, the occurrence of an increase upon black was far less evident.

The studies of Sumner and Fox (1933, 1935, 1935a) relate to quantitative changes in yellow pigment of the xanthophyll series. Fishes of several species (*Fundulus parvipinnis*, *Gillichthys mirabilis*, *Girella nigricans*) were employed in these studies, the xanthophyll being extracted with customary solvents and the resulting solutions assayed colorimetrically. While all these fishes respond in an imitative fashion to yellow backgrounds, as well as to black, white and gray ones, it is of interest that out of the three, *Girella* alone was found to manifest any differences in the actual amount of yellow pigment which it yielded. It is interesting, too, that the amount of xanthophyll present (though not the visible appearance of the fish) appeared to depend upon the shade rather than upon the color (spectral value) of the background. Thus, the greatest amount of xanthophyll was extracted from the almost inky black fishes which had been kept on black, the least from fishes kept on white, while intermediate and nearly equal amounts were yielded by specimens kept on a rather rich yellow and a neutral gray of medium shade. It should be added that *Girella*, in all circumstances, was found to lose xanthophyll under laboratory conditions.<sup>8</sup> The relations stated above indicate, therefore, relative rates of loss.

<sup>8</sup> This statement does not apply, however, to *Fundulus parvipinnis*.

In our studies, no attempt was made to count xanthophores, nor were any microscopic studies made. Abramowitz (1935), however, examined and counted xanthophores in the caudal fin of *Fundulus majalis*, before and after keeping the fishes on black, white, yellow and blue backgrounds. After periods ranging from twelve days to six weeks, increases were noted on yellow and black, decreases on blue and white.

Thus far, the quantitative differences which I have discussed have related entirely to the responses of the fishes, not to the stimuli which called these forth. The backgrounds have been characterized as "black," "white," "gray," etc., without reference to their exact degree of reflectivity nor to the intensity of the incident light. Several years ago the writer and one of his collaborators undertook to determine what quantitative relations might exist between various visual stimuli and the amount of melanin which could be extracted from fishes which had been subjected to these.

To measure these visual stimuli, at least in relative terms, was, of course, fairly simple. To measure the amounts of melanin, even approximately, was not so simple. However, after many months of experimenting, we developed a method which seemed reasonably adequate, and this was applied to a rather considerable series of fishes (Sumner and Doudoroff, 1937).

The fishes (gobies) were kept in glass aquaria, painted on the outside with black, white and two shades of gray. Sets of aquaria thus painted were exposed to two widely different illuminations, these last being in the ratio of approximately 40:1.

After the fishes had been submitted, night and day, to these conditions for nearly three months, an assay of the melanin was undertaken. It was found that the intensity of the illumination played a probable though a minor part in determining the amount of melanin produced (or retained), but that the chief factor in influencing this amount was the shade of the background. Of chief interest was

the fact that the series of melanin values, at least those from the more brightly lighted set of fishes, assumed a logarithmic rather than a linear arrangement when plotted against the albedos of the backgrounds (Figure 1). The

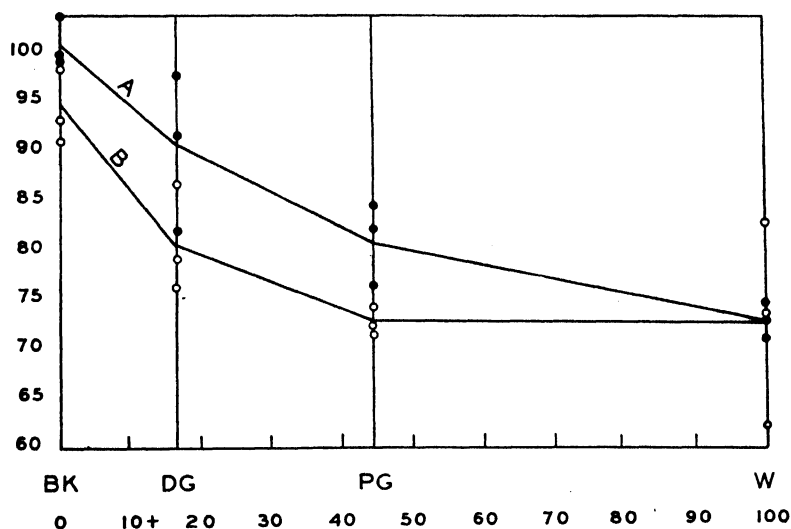


FIG. 1. Curves showing the mean relative melanin values of four lots of fishes (*Gillichthys*) from each of two cabinets. Solid circles represent those from cabinet "A"; open circles those from cabinet "B." Abscissas represent albedos, counting the albedo of the white bowls as 100 per cent. Ordinates represent relative melanin values, taken as percentages of mean value of "black" fishes, regarded as 100. (Modified from Sumner and Doudoroff, 1937).

significance of this last fact will be discussed after I have recorded some confirmatory evidence.

Our experiments with *Gambusia*, more recently reported (Sumner and Doudoroff, 1938), warrant but a brief mention here, owing to the lack of sufficient precision in our determinations. As a result, doubtless, of unperceived variations in our procedure, considerable differences were found between series of fishes which should have given identical results. It deserves reporting, however, that the graphs for the background experiment show a trend similar to that which we encountered in our experiment with gobies, i.e., a more nearly logarithmic than linear arrangement of the values (Fig. 2). On the other hand, no sig-

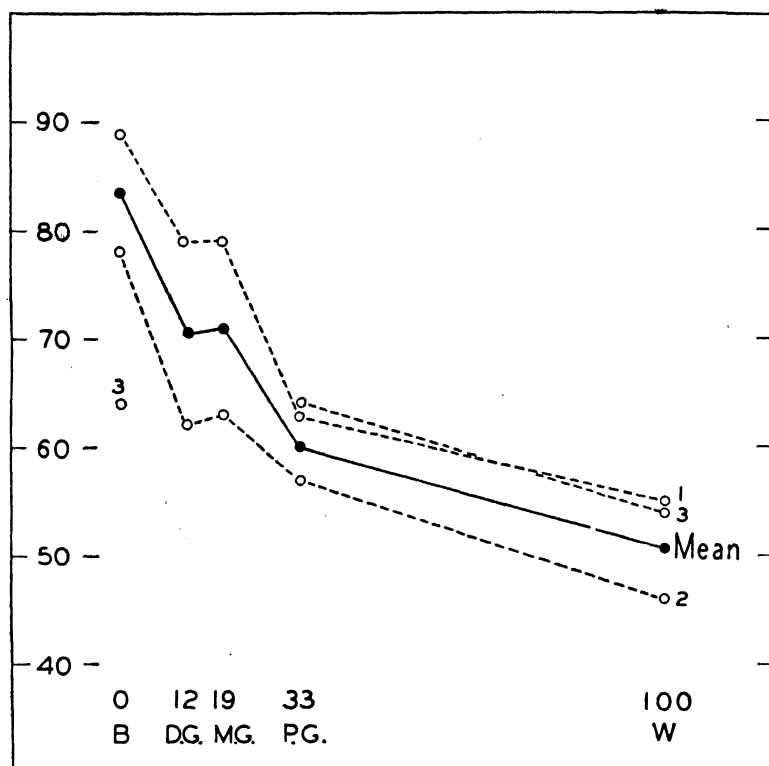


FIG. 2. Computed melanin content of fishes (*Gambusia*), exposed for two months (+) to backgrounds of various albedos. Abscissas represent albedos, counting white as unity; ordinates the melanin content, expressed in milligrams per 100 grams of original weight of fishes. The "mean" curve is based upon the means for series 1 and 2 (From Sumner and Doudoroff, 1938).

nificant differences were detected as a result of the widely different illuminations employed.

It became evident to us that any further effort to assay the melanin of fishes should be deferred until we had overcome certain difficulties of technique. Pending such action, we started a new experiment, this time on *Lebistes*, with a view to counting the melanophores in given unit-areas of the skin.

These fishes were kept in sufficient numbers in bowls of five shades (white, black and three grays) and exposed to light of an intensity of about thirty foot-candles. After

rather more than two months, they were subjected to adrenalin, the effect of which is to bring about the aggregated condition of the melanin and thus to facilitate the counting of the melanophores. The fishes were next fixed, decalcified and cleared in cedar-oil.

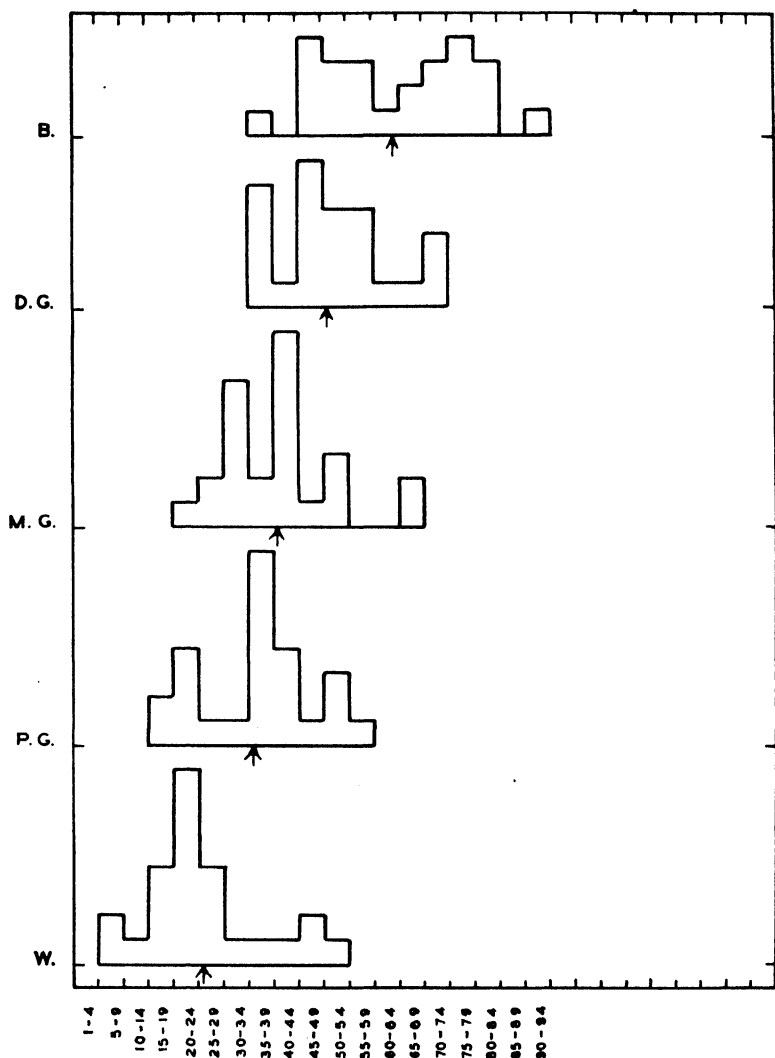


FIG. 3. Frequency distributions of number of melanophores in a given area of skin in premaxillary region of *Lebistes*. Twenty-five individuals from each of five backgrounds: white, pale gray, medium gray, dark gray and black.

Evaluation of results from this experiment is still under way. Using a suitable micrometer grating<sup>9</sup> in the ocular, I have already counted the melanophores in two regions of the skin of twenty-five fishes from each of the five series. Despite high individual variability, the mean differences among these five series appear to be quite significant. (Figs. 3, 4, 5). Most important of all is the defi-

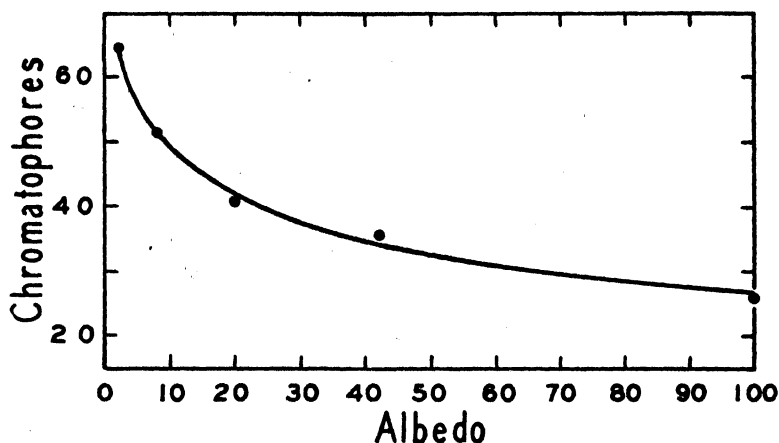


FIG. 4. Mean values of melanophores of the five lots of fishes shown in Fig. 3, plotted against the albedos of the backgrounds, white being regarded as unity.

nately logarithmic arrangement of the mean values for chromatophore number when plotted against the albedos of the background.

From these facts and others which I cited earlier, it would seem that increments of melanin production vary inversely as the logarithms of the increments of reflectivity of the background.<sup>10</sup> The experimental psychologist has long been familiar with the fact that for some of our senses, at least, and within certain limits, our increments of perception are proportional to the logarithms of the increments of the stimulus (the "Weber-Fechner law"). I believe that our analogy here is more than a

<sup>9</sup> I had employed this method in a preliminary experiment upon *Fundulus parvipinnis* in 1931 (results not published).

<sup>10</sup> Or *vice versa*, the decrements of each.

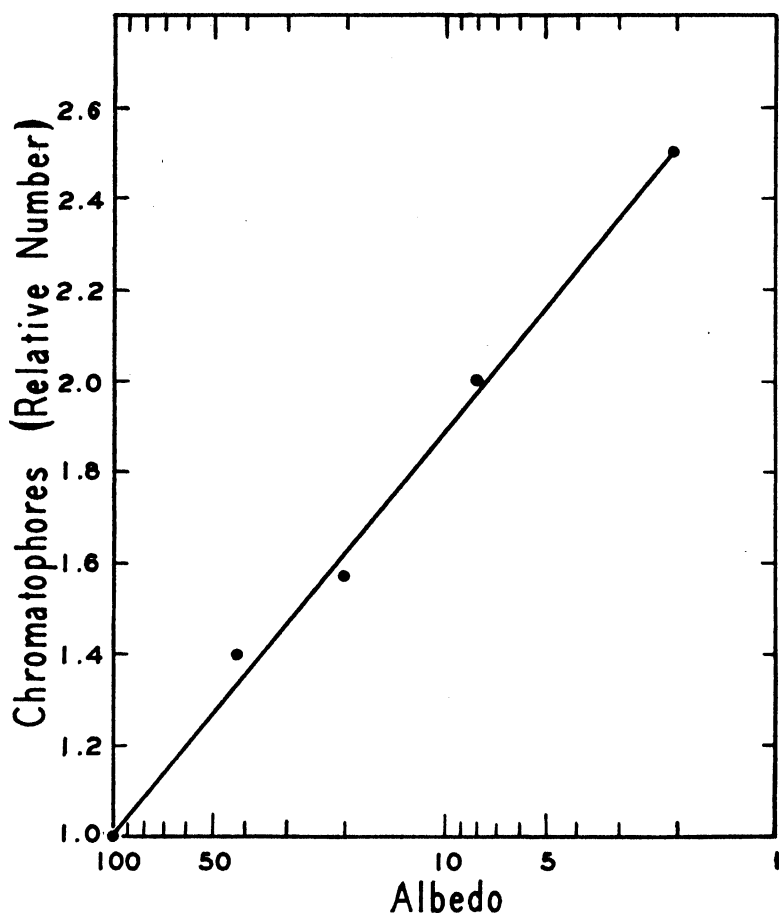


FIG. 5. Mean values shown in Fig. 4, plotted against the logarithms of the albedos. (The albedo of "black" has here been regarded as 2 per cent.)

superficial one. I shall refer again to this case in my address before the Zoologists to-night. (In press, *Scientific Monthly*).

A few words in conclusion regarding this matter of backgrounds. I have spoken glibly of a fish's responding to the albedo of the background, as if a background of a given albedo provided an optic stimulus of constant value. This, of course, is far from the case. The albedo of a surface denotes the proportion of incident light which that surface reflects. The absolute amount of reflected light



varies enormously with the source of illumination. And yet we, in our perceptions, and the fish, in its pigmental responses, appear to recognize the albedo at its true value, largely irrespective of the intensity of the incident light. Both of us, in some way, make allowance for this last.

In 1911, I expressed the view that it was the ratio between the light received from overhead and that reflected by surrounding and underlying surfaces which was chiefly influential in determining the shade assumed by the fish.<sup>11</sup> This view, as it happened, had been expressed in closely similar language by Keeble and Gamble (1904) for certain crustacea.

It may be, therefore, that the upper and lower halves of the field of vision influence the animal in an opposite sense. That the retina of a fish is in some way physiologically polarized is an obvious thought. Attempts to test this hypothesis have been made by several workers. By covering the eyes of *Fundulus parvipinnis* with false corneas of celloidin, variously divided into clear and darkened areas, I obtained some evidence for this view (Sumner, 1933).<sup>12</sup>

Butcher and Adelman (1937), Vilter (1937) and Butcher (1938) have reported experimental results which point to the same conclusion from rotating the eyeballs of fishes on their axes through an angle of 180°. My own efforts to perform this experiment had been unsuccessful, owing to the drastic procedure involved. However, it seems far from certain that the fish's response to the light relations within its field of vision is regulated entirely by any such

<sup>11</sup> My earlier discussions referred to physiological color changes only. An experimental demonstration of the above proposition was made by Sumner and Keys (1929), and these findings were confirmed by Pearson (1930) for young *Ameiurus melas*. Brown (1936), using more exact quantitative methods, reached the same conclusion for the fish *Ericymba buccata*, except for very low illuminations.

<sup>12</sup> von Frisch (1911) had attempted a similar experiment by applying an opaque paste to the eyes. Butcher and Adelman (1937) and Butcher (1938) have reported extensive confirmatory results from experiments of this type.

simple polarity of the visual mechanism. It is not true, of course, of our own perception of differences of shade.

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# THE RESPONSES OF MELANOPHORES IN ISOLATED FISH SCALES

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THERE are among the vertebrates the following types of effector organs: muscles, glands, cilia, chromatophores and retinal pigment cells. Of these the chromatophore is the only one not found in all groups. Its distribution is limited to the reptiles, amphibians and fishes. Among the chromatophores themselves many different kinds are known, distinguished from each other by the pigment they carry. Of all these perhaps the easiest to study is the fish scale melanophore. This is because the scale is easily removed from the fish without injury to the melanophores, and the scales being translucent the melanophores are readily visualized under the microscope.

The obvious response of the melanophore to stimulation is a change in the distribution of its pigment granules. In Fig. 1, taken from a paper by Spaeth (1913), are shown three different stages of melanin aggregation. The first (a) is conveniently known as the concentrated, contracted or punctate state, the second (b) as the stellate state and the third (c) as the dispersed or expanded state. From time to time various attempts have been made to describe the shift from one state to another. These range all the way from simple visual estimation of the amount of pigment migration to methods for measuring the length of the melanophore process with a micrometer scale. Recently (Smith, 1936) I have succeeded in following these movements with a photoelectric cell by measuring the amount of light transmitted through the scale, which in turn is determined by the state of pigment distribution of

<sup>1</sup> Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.

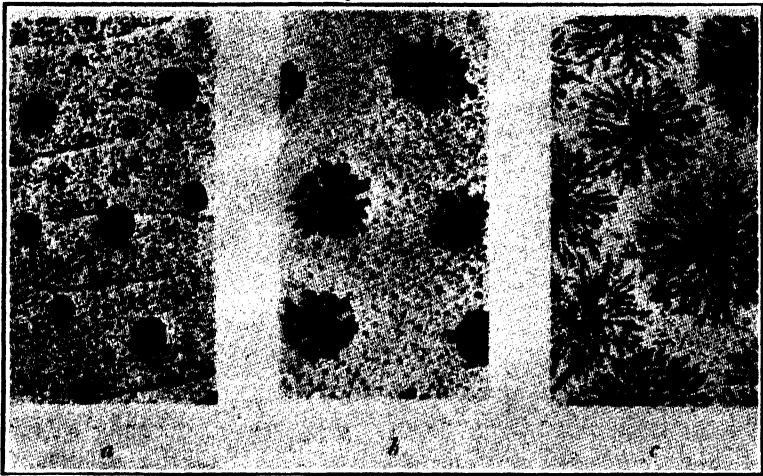


FIG. 1. Isolated scale melanophores of *Fundulus heteroclitus*. a, Concentrated or punctate state; b, stellate state; c, dispersed state. R. A. Spaeth, *Jour. Exp. Zool.*, 15: 527, 1913.

the melanophores. This method has proven reasonably successful.

It will be quite impossible to do more than discuss briefly the effects of some of the more important types of stimulation upon melanophores. These will be their responses to (1) variations in the electrolyte concentration of the medium, (2) changes in the amount and kind of radiant energy falling upon the cell, (3) responses to the presence of various drugs and (4) a short discussion of melanophore pulsations. In all this work the scales were removed from the trunk of the fish and their melanophore responses observed directly under the microscope.

#### ELECTROLYTES

Spaeth (1913) was the first to describe the effects of various electrolytes on pigment distribution. Very briefly his results may be summarized as follows. In solutions of sodium salts, as shown in Fig. 2, No. 6, the melanophores are completely dispersed. This dispersion lasts about three hours and is followed by a slow concentration. This last is a sign of the approaching death of the cell.

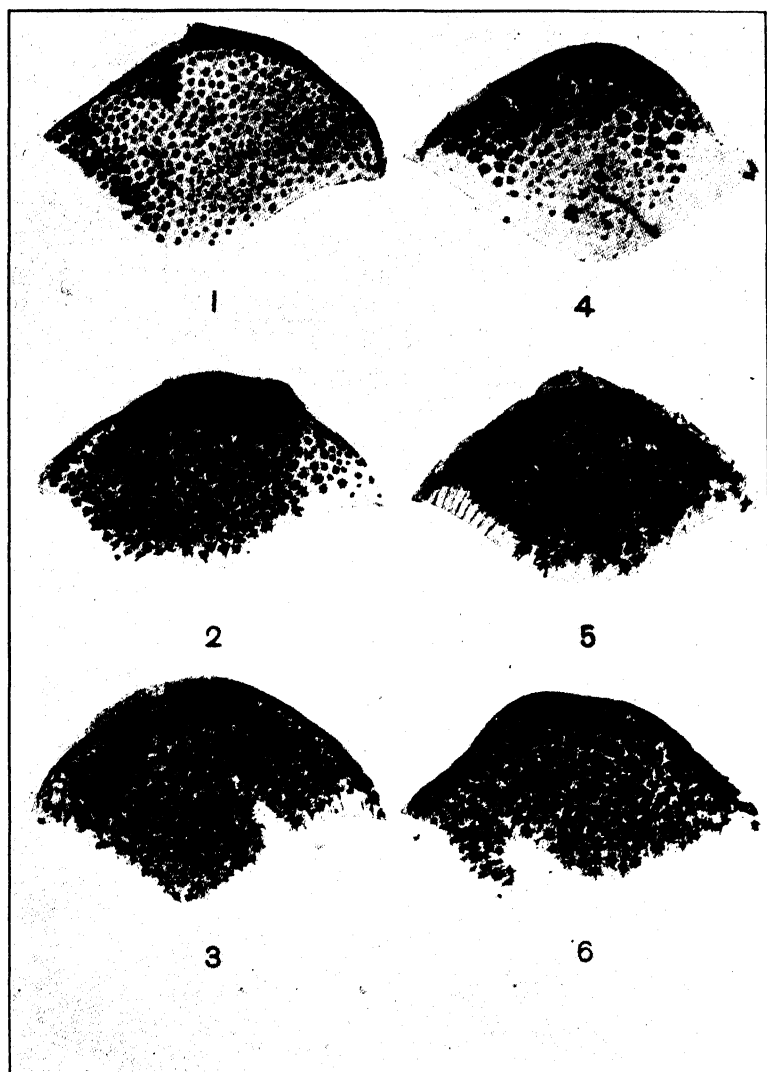


FIG. 2. Isolated scales of *Fundulus heteroclitus*, showing melanophores in different states of pigment aggregation. R. A. Spaeth, *Jour. Exp. Zool.*, 15: 527, 1913.

Spaeth found the expansion to persist longest in NaCl and to become progressively shorter as the anion shifted from Cl<sup>-</sup> to Br<sup>-</sup> to No<sup>3-</sup> to SCN<sup>-</sup>.

In solutions of potassium salts the melanophores become

completely concentrated, the scale resembling in appearance No. 1 in Fig. 2. The concentration lasts three or four hours and then is gradually replaced by a slight dispersion, again a sign of approaching disintegration. The period of concentration depends upon the anion and is longest in KCl, becoming progressively shorter as the anion is changed from Cl- to Br- to NO<sup>3-</sup> to SCN<sup>-</sup>.

As is to be expected, sodium and potassium salts antagonize each other, and the melanophores retain their irritability longer in a mixture of the two than they do in either one alone. As an artificial medium for maintaining melanophore irritability Spaeth found the best to be made up as follows: 6 vols. N/10 NaCl, 1 vol. N/10 KCl and 0.35 vols. N/10 CaCl<sub>2</sub>. This he called a "balanced solution," and with some modifications it has been used by most subsequent investigators.

#### RADIATION

Spaeth (1913) was also the first to show that on exposure to ultra-violet light (1,900–2,900 Å) the melanophores of isolated *Fundulus* scales showed a concentration of their pigment. This is shown in No. 1, Fig. 2. No such concentration occurs in control scales kept in NaCl nor in scales so placed that their bony portions lie between the melanophores and the light source (Fig. 2, No. 2). Apparently the radiation is absorbed in these cases before it gets to the cell. It takes from 7 to 10 minutes to complete the response and it is reversible in 20 minutes. The first cells to concentrate are those lying in the middle of the scale, and from here the reaction spreads towards the periphery. Spaeth's observations were later corroborated by O'Donnell (1927).

Spaeth, on the other hand, was unable to demonstrate any response to visible light in *Fundulus* scale melanophores. This is generally the case with most fishes. Some time ago, however, I was fortunate to observe a rapid dispersion of the melanophore pigment in *Tautog* scales when the light intensity was suddenly increased.

Fig. 3 shows this response, the scale having been kept for varying periods in relative darkness. The recording is by the photoelectric cell method previously described.

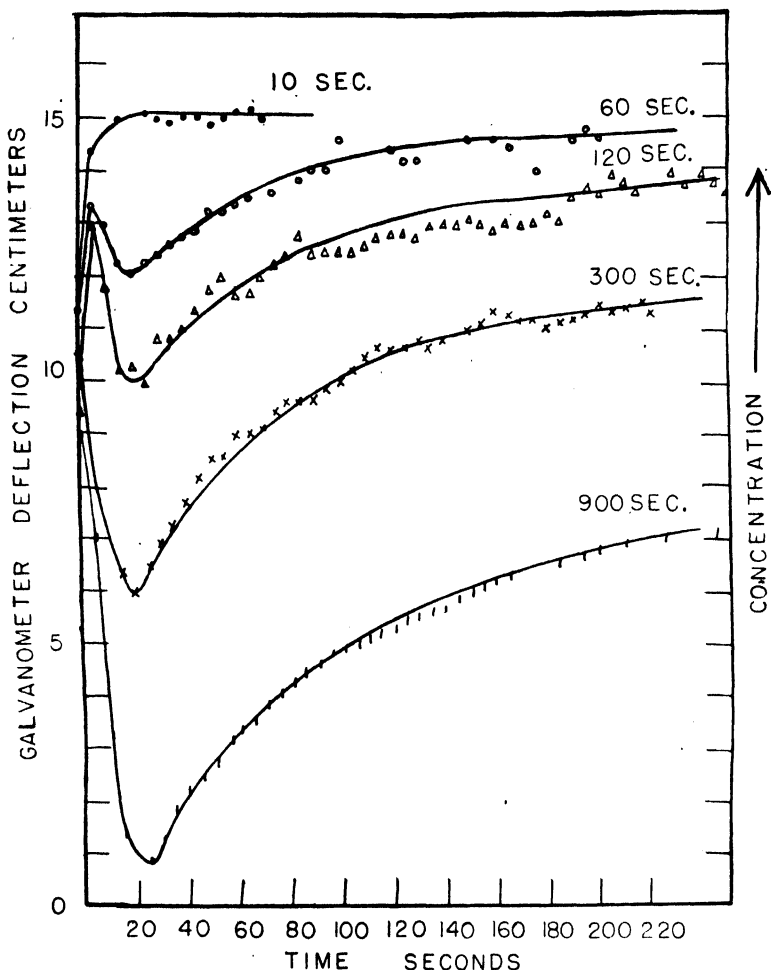


FIG. 3. Isolated scale melanophores of *Tautoga onitis*. Responses to sudden increase in illumination after varying periods in relative darkness. Photoelectric recording.

As can be seen from the figure, the dispersion is complete in about 30 seconds. Following this there is a slow return to the stellate or the concentrated state. The scales were kept in balanced solution throughout the experiment.



The extent of the dispersion seems to be proportional to the length of time the melanophores were dark adapted. Under the conditions of the experiment the response must be direct, since in an isolated scale there can be no question of circulatory or nervous mediation. It is my opinion that this response in Tautog scales is a persistent form of juvenile activity which is not retained in most fishes. No doubt it will be found in a few other forms if a search is made, although it definitely is not present in *Fundulus* scales.

Contrasting the responses of the scale melanophores to ultra-violet light and visible light, we note several marked differences. In the first place ultra-violet light produces a concentration and visible light a dispersion of the pigment. Secondly, the speed of the response varies greatly, being slow in ultra-violet light and very rapid in visible light. Finally in the irradiated scales the response appears first in the center and spreads outward, while in visible light the response occurs simultaneously throughout the entire scale. All this suggests that in the case of ultra-violet light the response is due to some chemical change in the tissues brought on by the radiation, while in the case of visible light it is due to direct stimulation of some presumably photosensitive material in the melanophore.

#### DRUG ACTION

It must not be assumed, however, that all responses of isolated melanophores are non-nervous in nature. There is the possibility that stimuli may act upon the torn ends of the severed nerves or upon the chromo-neural transmitting apparatus. The only way to exclude definitely the possibility of nervous intervention is to study the responses in scales in which all nervous structures have been eliminated. Following a method originally developed by Dr. H. B. Goodrich, such preparations may be obtained as follows. The scale is pulled from its scale pocket and all its connections with the body are severed. It is then immediately replaced in the pocket and in the major-

ity of instances it remains there. However, in conformity with the laws of nervous degeneration all the nervous structures in the scale should disappear in time. To see whether there are any changes in the melanophore responses which could be correlated with this nervous degeneration the reactions of the cells were tested from time to time to various stimuli. The most successful were those concerned with drugs.

Fig. 4 shows the results of such a study on the effects

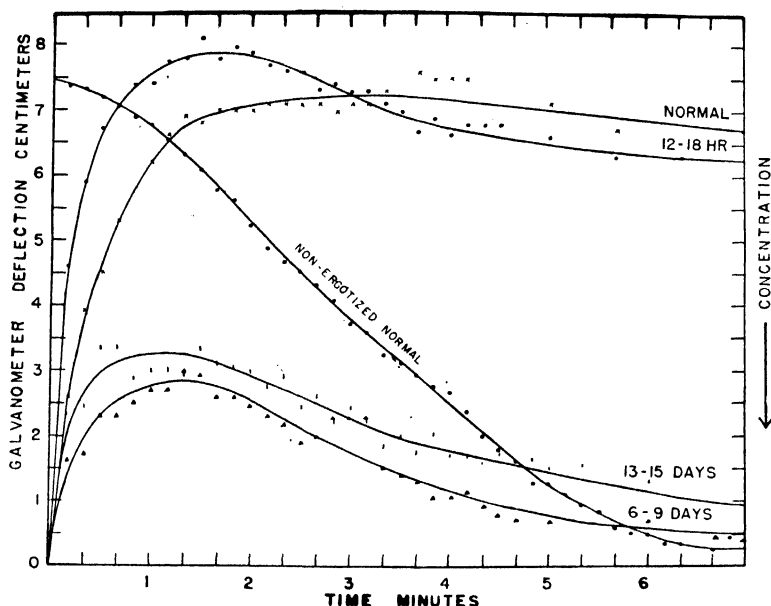


FIG. 4. Isolated scale melanophores of *Tautoga onitis*. Responses to adrenalin after previous treatment with ergotamine. Time after denervation of scale shown by figures above right-hand end of each curve. Photoelectric recording.

of adrenalin, both alone and after previous treatment of the scales with ergotamine. Adrenalin alone produces a marked concentration of the pigment. The figure shows such a response in a normal scale as recorded photoelectrically. No appreciable differences between the responses in normal scales and those taken at various times after denervation could be seen.

Such, however, is not the case with scales previously

treated with ergotamine. Spaeth and Barbour (1917) made the interesting discovery that after ergotoxine adrenalin expanded instead of contracted the melanophores. The same reversal may be produced in the Tautog scale with ergotamine, as shown in the figure. In about 24 hours after denervation, however, the character of the response is markedly altered and remains that way for at least fifteen days. While some dispersion still occurs, it is only a fraction of what it formerly was, and it persists for a relatively short period of time. Clearly the nervous degeneration going on in the scale has affected

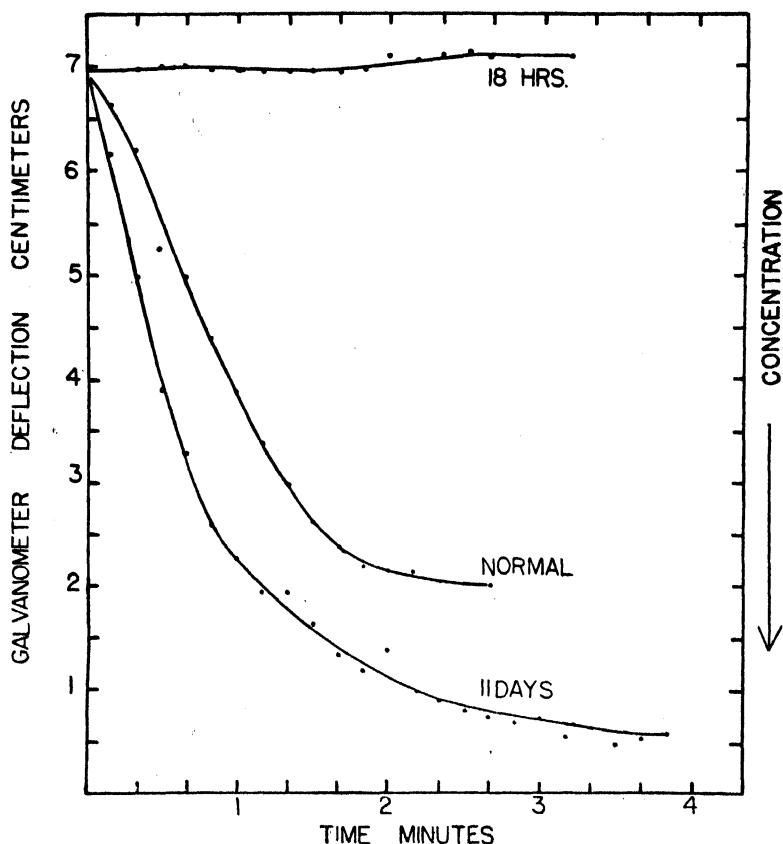


FIG. 5. Isolated scale melanophores of *Tautoga onitis*. Responses to N/5 KCl of normal scale and scales tested 18 hours and 11 days after denervation. Photoelectric recording.

some structure acted upon by ergotamine, which in turn is responsible for the reversal of the adrenalin reaction. Adrenalin alone still produces concentration at this time. In fact the only difference between this and the response to adrenalin after ergotamine is a slight momentary dispersion in the latter case. We may conclude from this that adrenalin acts at some point peripheral to the action of ergotamine. Since the ergotamine-adrenalin response is altered by nervous degeneration we may conclude that the response to adrenalin alone is direct. If adrenalin is the mediator of sympathetic impulses, as many think, such a result is not surprising.

Next the effects of KCl were studied. This also produces a marked concentration, as shown in Fig. 5. This concentration no longer occurs in about 18 hours after denervation, but reappears again in about 11 days. The concentration produced by KCl then depends upon the integrity of some structure associated with the innervation of the scale. From this we may conclude that KCl does not act directly upon the cell, but rather upon the chromo-neural transmitting apparatus, and therefore its action is proximal to that of adrenalin. Possibly KCl acts to release from the transmitting apparatus some adrenalin-like substance which in turn causes the pigment concentration.

#### PULSATIONS

Finally, in conclusion I want to mention briefly a very interesting type of response shown by certain chromatophores. These take the form of pulsations of the pigment granules and were first described by Ballowitz (1913) as occurring in the erythrophores of *Mullus* when immersed in NaCl. Fig. 6 shows the two extremes of pigment migration exhibited during these pulsations. The figure is taken from the original description by Ballowitz. We have also described similar pulsations in the scale erythrophores of the squirrel fish, *Holocentrus* (Smith and Smith, 1935). Spaeth (1916) quite independently observed a similar phenomenon in the scale melanophores of

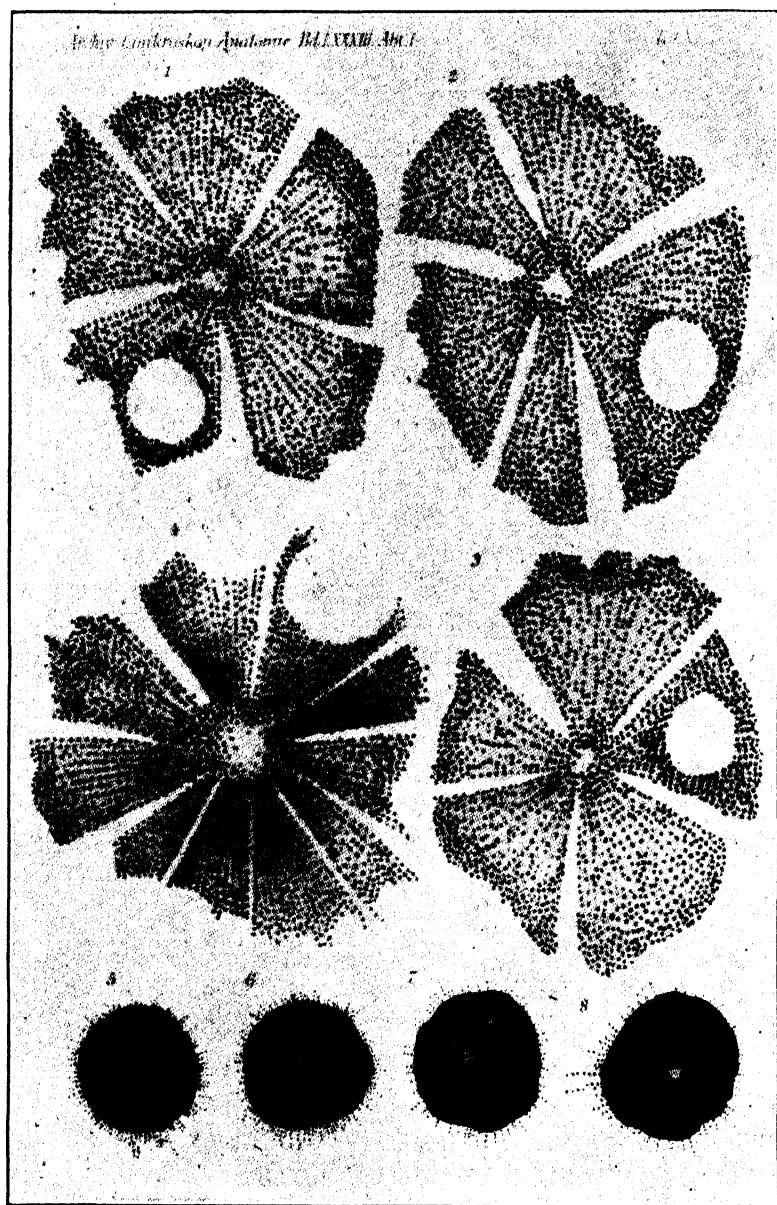


FIG. 6. The extremes of pigment distribution in the isolated erythro-  
phores of Mullus during pulsations. E. Ballowitz, *Arch. mikro. Anat.*, 83:  
290, 1913.

Fundulus immersed in NaCl after previous treatment with BaCl<sub>2</sub>. Fig. 7 shows the frequency and extent of these

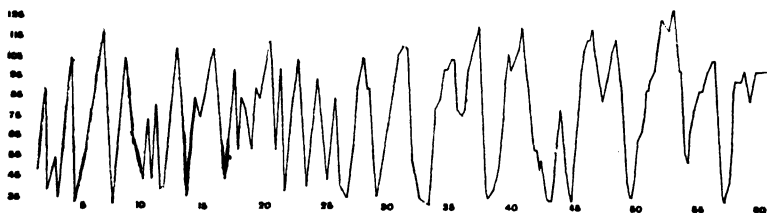


FIG. 7. Rhythmical pulsations in the isolated scale melanophores of *Fundulus heteroclitus* immersed in N/10 NaCl after previous treatment with N/10 BaCl<sub>2</sub>. R. A. Spaeth, *Jour. Exp. Zool.*, 20: 193, 1916.

pulsations as measured with a micrometer scale. These pulsations have been shown to vary in frequency and extent with the temperature, being more rapid and very slight at high temperature (30° C.) and much slower but with pigment migration over a greater distance at low temperatures (Smith, 1931).

Similar pulsations have also been seen in the scale melanophores of the Tautog. Their extent and frequency as measured with the photoelectric method is shown in Fig. 8.

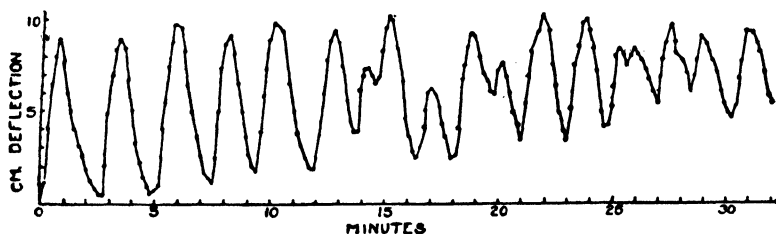


FIG. 8. Rhythmical pulsations in the isolated scale melanophores of *Tautoga onitis* (L.) as recorded photoelectrically. D. C. Smith, *Jour. Cell. Comp. Physiol.*, 8: 83, 1936.

Parker and Pumphrey (1936) showed that in *Fundulus* these pulsations were not dependent upon the presence of any nervous mechanism in the scale, and the same is undoubtedly true for other forms.

The nature of these pulsations is still obscure. They may be associated with some electrolyte disturbance in the cell. Yamamoto (1933), who studied them in the scales of

Oryzias, believes they are produced by an excess of sodium over calcium ions. In any event they are worth studying further, for they undoubtedly are an expression of the underlying forces producing pigment migration.

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## HUMORAL CONTROL OF CRUSTACEAN CHROMATOPHORES\*

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MANY higher crustaceans are able to change their color and pattern by activities of their chromatophores to accord more or less perfectly with the background upon which they reside. Generally speaking, the activities of the chromatophores in color changes are of two sorts: differential formation and destruction of the included pigments, and differential migration of the pigments within the chromatophores. This discussion will deal only with the controlling agencies of the pigment migration, but even with this restriction it will be impossible to include more than the principal facts, with the chief evidences for them.

The descriptive aspects of crustacean color changes were well advanced through the investigations of such men as Pouchet (1872-1876), Gamble and Keeble (1900-1905), Megušar (1908) and Degner (1912). These investigations were conducted during a period when the control of the color changes was believed to be wholly nervous. Complexity of response was then easily explained away in terms of central nervous phenomena. Degner had come very close to the modern concept of humoral control of chromatophores when he discovered that nerve transection failed to interfere with the chromatophore responses.

The real steps that revolutionized our outlook on the general problem came in 1925 and 1927 when Koller discovered that blood transfusions carried with them a substance or substances capable of inducing chromatophore response. In 1928 Perkins, working on *Palaemonetes*, and Koller on *Crangon septemspinosus* (Say) independently reported that eyestalk extract had a very potent

\* Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.



effect in inducing chromatophore pigment concentration. Perkins further demonstrated quite conclusively by means of exhaustive nerve transection experiments that the normal responses of the chromatophores to black and white backgrounds were in no way effected by direct nerve supply, but were totally controlled by blood-borne material. Koller, on his part, described the rostral region as a source of a second extract which worked antagonistically to the eyestalk substance.

Let us now look briefly at the pigmentary system and its activities to see just what we are attempting to explain. Crustaceans generally have three to five functional pigments within this system. The combination red, yellow, blue and white is common among shrimp. In certain other crustaceans a black or brown-black pigment is present.<sup>1</sup> Work already described has demonstrated the gross chromatophore control to be humoral. The extent of independent activity of the individual pigments will indicate to us the complexity of the situation which must be explained in humoral terms. Speaking of pigments, Keeble and Gamble (1900) say, "During colour-change they are distributed independently of one another in the sense that one pigment may become aggregated in the center of the 'chromatophore,' whilst another runs out into its network of processes. Change of colour appears to be due to a fresh pigmentary deal of the shuffled colour pack." Pigment independence in Hippolyte was confirmed by Minkiewicz (1908). Koller (1927) describes independence of the red and yellow pigments of Crago. Brown (1933, 1935a, 1935b) demonstrated that each of the four pigments of Palaemonetes was able to show independent activity. For all possible combinations of two pigments at a time, different experimental situations illustrated that one pigment could be dispersed or concentrated while the other pigment was simultaneously in the same or opposite state. Frequently the same pigment cells in different experimental situations were ob-

<sup>1</sup> This classification of pigments by color does not necessarily indicate chemical similarities.

served during several days and photographic records made demonstrating such independence. Observing the same pigment cells in such an experiment precludes the possibility that an apparent independence arises from the selection of different cells with different normal responses. More recently Abramowitz' (1935) data have demonstrated for the crab, *Portunus ordwayi*, an independence of the red and yellow cells.

Another interesting general behavior of the chromatophores should be stressed. There is a striking tendency for all the pigments in a crustacean, like *Palaemonetes*, to behave rather uniformly relative to one another. That is, in the great majority of the immediate responses of the chromatophores the red and yellow pigments concentrate or disperse together while the white usually migrates in the opposite direction. If one considered only this aspect of color change there would be but little evidence of an independent behavior of the various pigments, and the action of a single humoral substance could account for the whole. The delicate differential control of the pigments which occurs in the animal in response to the background color makes its appearance only after a period ranging from fifteen minutes to more than a day. It is this secondary migrational response that demonstrates the independence of the pigments. The investigations of Keeble and Gamble, Koller and Brown have all given indications of these two distinct types of pigmentary response. It would perhaps seem that the rapid initial response is determined by apparent brightness of background or change in total illumination. The secondary response of the pigment cells has apparently been overlooked by many recent investigators of crustacean chromatophore control. By finding quite normal responses in denervated areas of the body, Brown (1935b) showed that the secondary pigment migration demonstrating independent activity of the pigments was humorally controlled.

The recent extensive comparative surveys of color-changes and eyestalk-extract injection effects have con-

tributed relatively little to our understanding of the controlling mechanism of the intricate color adaptations in any single crustacean. They have, however, given strong suggestion that eyestalk-extract is qualitatively quite uniform regardless of its source among crustaceans.<sup>2</sup> It has demonstrated, furthermore, that chromatophores of the same color in different crustaceans may be inherently quite different in their responses. To the same eyestalk extract sample the red pigment of a brachyuran response by dispersion and that of a macruran by concentration. Until chemical isolation and purification of the constituent which induces concentration in one red pigment cell has been effected it is impossible to say definitely whether the same constituent or a different one is responsible for the dispersion in the red cell of another animal.

Hanström (1935, 1936) has presented good evidence that the active portion of the eyestalk in chromatophorotropic activities is the so-called sinusgland. This is composed of distinctly glandular tissue,<sup>3</sup> richly innervated, and present in all crustaceans so far examined.

No such general confirmation has been established concerning the rostral gland first described by Koller (1928, 1930); but in 1934 Beauvallet and Veil obtained data suggesting its presence in *Palaemon*. Although I am not prepared to give evidence in favor of a functional rostral gland, I do have some observations upon *Crago septemspinus* which indicate a definite difference in the control of some of its chromatophores from that found in *Palaemonetes*. The observations were as follows: *Crago* and *Palaemonetes* failed to respond in the same manner to removal of both eyestalks with their included glands. Although *Crago* and *Palaemonetes* both darkened maximally at first, *Crago* finally assumed a mottled condition while *Palaemonetes* remained dark. Artificial stimulation of the stubs of the excised stalks produced transitory

<sup>2</sup> This may not be true from the standpoint of retinal pigment controlling hormones (Kleinholz, 1936).

<sup>3</sup> The cells show eosinophilic cytoplasmic inclusions.

darkening in Crago and transitory lightening in *Palaemonetes*. In another experiment, occluding the blood supply to the eyestalks and rostral region resulted in rapid darkening in light-adapted *Palaemonetes*, but almost no darkening in Crago, during the hour following the occlusion. These observations find a reasonable explanation in the activity of a dispersing hormone, perhaps of rostral origin, in Crago and the absence of one in *Palaemonetes*.

There is no evidence beyond these few cases already mentioned suggesting the presence of a humoral substance operating antagonistically to the eyestalk extract. On the contrary, in the majority of the crustaceans examined, there is evidence pointing to the absence of such substances. Procedures rendering the eyestalk glands ineffective are conducive to chromatophores assuming a condition opposite that which has been shown to be the result of an activating humoral substance. For example, general red and yellow pigment dispersion follows removal of the eyestalks, or occlusion of blood supply to the eyes. Cutting an artery results in pigment dispersion in the region devoid of blood supply. Isolation of pieces of integument results in similar behavior of the isolated pigments. A very significant experiment was performed by Perkins and Snook (1931), who produced repeated concentration and dispersion in the red and yellow cells of an isolated piece of integument merely by adding eyestalk extract. The chromatophores showed dispersion of their pigments as the effectiveness of the extract disappeared. Even the rates of dispersion resulting from isolation are approximately those seen in normal responses (Brown, 1935b).

There are a number of indications of the production of chromatophorotropic substances in regions of the body outside of the eyestalks. Such evidences come from injection and eyestalk extirpation experiments. Injections of extract of central-nervous-organs induce concentration of shrimp red and yellow pigments. This was independently shown by Brown (1933) and Hosoi (1934). The activity of the extract is independent of the state of adaptation of

animals from which the extract was made. Eyestalkless crustaceans show activity of their chromatophores. This activity may be random as with the white cells of some forms, in response to artificial stimulation of the eyestalk stubs, or in response to changes in light intensity. By means of such experiments and study of the effects of injection of the crude extracts of eyestalks we are led to the conclusion that the chief source of the red and yellow pigment controlling agents is in the eyestalk, while the chief source of the white pigment controlling substance is outside of the eyestalk. Further evidence of the extra-eyestalk origin of pigment controlling substances comes from study of the quantitative effects of the potency of single stalks as compared with two stalks in the normal responses.

Except in the instance of *Crago*, where antagonistic hormones may control a single pigment, a single type of effect of an activating substance upon a pigment seems to be the rule. Originally we believed this effect to be always pigment concentration, but since the work of Carlson (1935) we know that it may be either pigment concentration or dispersion, depending upon the species. The reverse response appears to result passively from the disappearance of the activating substance.

To account for the apparent independence of pigments Brown (1935b) has postulated the existence of a separate hormone for each pigment of *Palaemonetes*. This seemed to be the most reasonable explanation in that it requires the fewest hormones. If one assumes that eyestalk extract contains a single homogeneous chromatophore activator, then one would need to postulate the addition of a minimum of four "modifiers" to explain the normal pigment independence in color changes. An interesting speculation may be advanced here, namely, that the sinusglands secrete a chromatophorotropic substance the molecules of which are subject to modification of superficial structure through nerve control. Such molecules might find their most stable form or equilibrium proportions in the condition seen in eyestalk extracts.

The rich nerve supply to the sinusgland from the lamina terminalis described by Hanström is interesting in connection with any one of these hypotheses. Suggestive also is the condition of eyestalk extract in such forms as *Cambarus* in which the chromatophorotropic activity is greatly enhanced by boiling. It is possible that boiling may remove inhibitors of chromatophorotropic activity. A comparable but more selectively inhibiting mechanism might be operative in the "four-modifier" hypothesis mentioned before.

Abramowitz (1937) speaks of the hypothesis of the system controlled by several hormones as the "multiple theory." He proposes as a possible alternative a "unitary theory" and states that it explains the situation equally well. The unitary theory is contradicted by certain facts. For instance, it has been shown that the red and yellow pigments may show degrees of concentration and dispersion quite independent of one another. To explain this in terms of a single hormone would require differences in threshold of response for the two pigments, and furthermore, there would need to be some higher concentration of the hormone which would simultaneously induce dispersion of one pigment and concentration of the other. Experimentally, no injection of eyestalk extract, if above the minimum strength required to concentrate both pigments, has resulted in dispersion of one of them. This has been true though very concentrated extracts have been used. Similarly, a unitary theory is even less able to explain the relatively independent behavior of four pigments. To do this last, some pigments must have not only a single threshold value of hormone concentration for activation but must have a number of alternately activating and non-activating values as the hormone concentration is gradually increased.

Since some chromatophores in a number of crustaceans appear to respond to a greater or less degree to direct light stimulation it might be suggested that this fact, together with a single hormone, may explain the color adaptations.

This is highly improbable, since blinded animals show no longer any trace of background response and also because the independent behavior of the pigments can be demonstrated in the dorsal trunk region cells where the illumination on the cells is almost exclusively the direct light in contrast with the background reflected light. This direct light may be maintained constant in quantity and quality in experiments demonstrating pigment independence.

Finally, we know nothing whatsoever of interactions and mutual effects of the various hormones operating in color changes. This alone could complicate the picture considerably.

Summarizing, it may be said that the crustacean chromatophore system contains four or five independently acting and humorally controlled pigments. These facts point to the functioning of at least four, and possibly more, hormones. These substances appear to be formed chiefly in the sinusgland of the eyestalks, though secondary sources may be present. The pigments appear to have one phase of their activity, concentration or dispersion depending upon the species, determined by action of a hormone, while the remaining phase appears to be the result of absence of the hormone. For only one crustacean, *Crago*, does evidence remain suggesting dual control of a pigment.

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# DISTRIBUTION OF INTERMEDIATE-WINGED APHIDS IN THE FAMILY AND ITS BEAR- ING ON THE MODE OF THEIR PRODUCTION<sup>1</sup>

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THE method of stimulation of wing development in aphids may presumably be either induction or the influence of some hormone or other chemical substance. Induction would involve the action of cells or tissues on other cells or tissues with which they are in contact or at least in very close proximity, and the spread of the stimulus should be relatively slow. A hormone could be in the body fluids, and so spread rapidly to any part of the organism, far as well as near.

As between these two methods, the conclusions of Stiles (1938) regarding the order of determination of the several distinguishing structures of winged aphids appear to favor the latter. Stiles obtained evidence that, of the three chief features, wings are determined first, then the ocelli, then the wing muscles. Wings get their start in some way not yet discovered. If the ocelli and wing muscles owe their stimulation to induction from the wings, that influence could either be a chain in which wings stimulate ocelli and ocelli stimulate wing muscles, or the wings could induce both ocelli and wing muscles directly, the former earlier than the latter. If induction is a chain, it acts in a roundabout way, spreading from wings to the more distant ocelli, then returning to the wing muscles, which closely adjoin the wings. If induction is a direct influence of wings on both ocelli and wing muscles, it spreads the greater distance to ocelli faster than the shorter distance to wing muscles.

Though these considerations are not conclusively against the induction theory, they render the hormone theory the

<sup>1</sup> Contribution from the Department of Zoology of the University of Michigan.

more probable. The hormone, while presumably produced at some one place, would quickly be everywhere, through circulation, ready to act as soon as the conditions of stimulation and of response were met.

How does this conclusion bear on the occurrence of intermediate-winged aphids? If it be assumed that the production or suppression of wings is dependent upon the presence of a hormone in a given concentration at some critical time in development, and if this substance may be accumulated at different rates or in different total amounts so that intermediate-winged individuals are sometimes produced, then it would be expected that some relation would exist between the frequency of intermediates and the frequency of fully winged aphids. Various possible mechanisms, whose operations would lead to the development of intermediates or mosaics or both, have been discussed in an earlier paper (Shull, 1937). A greatly simplified modification of the scheme will suffice for our present purpose.

The adopted scheme owes some of its characteristics to the discovery that all aphids of the species studied (*Macrosiphum solanifolii*) have thickened hypodermis in the wing-bud region in embryonic stages before birth (Shull, 1938). Even those which will be wingless as adults have wing buds before they are born. Something must stimulate that thickening. The wing buds disappear later in those that are to be wingless, but increase their thickness and begin to bulge in the prospective winged ones.

One possible assumption is that all embryos contain a hormone in sufficient concentration to stimulate observable thickening of the hypodermis. A greater concentration is needed, however, to cause further growth, and this concentration must be present shortly before birth when wings are finally determined. Only in certain individuals or under certain conditions is the concentration thus increased early enough; hence some aphids remain wingless. Figure 1, A, illustrates this scheme. If in some individual the concentration of the hormone reaches a level between

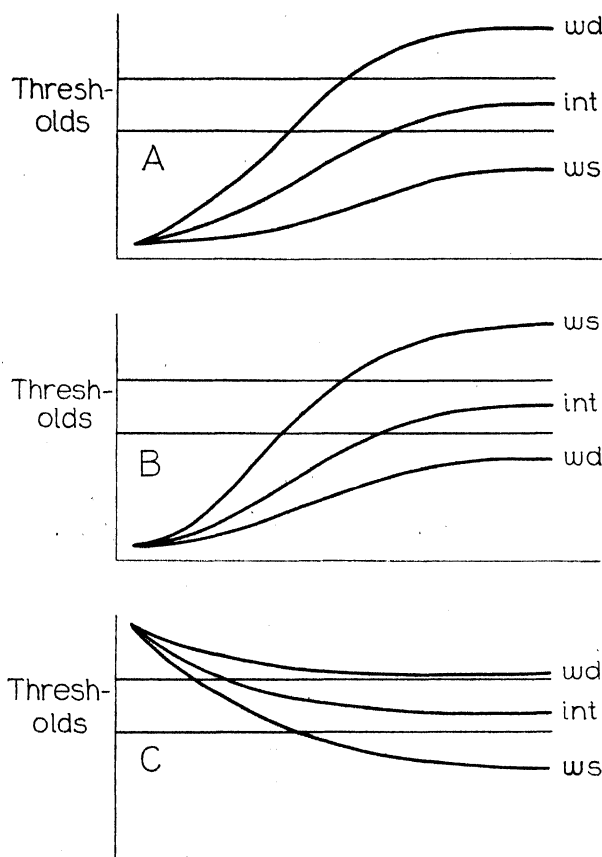


FIG. 1. Diagrams of several possible schemes by which wing development may be stimulated or inhibited by a hormone. The heavy curves represent the concentration of the hormone. The light horizontal lines represent the concentrations which will stimulate beginning development (lower) and complete development (upper), respectively. A, stimulating hormone; B, inhibiting hormone; C, stimulating hormone which decreases in amount with age.

that sufficient to stimulate the beginning of wing development and the amount necessary to cause complete development, intermediate wings are produced. The existence of intermediates necessitates the assumption that there is a gap between these thresholds of stimulation or that determination of wings is spread over a time interval. The latter possibility is not illustrated in this diagram, but was considered in the earlier paper (Shull, 1937).

Another possibility is that the hormone is inhibitory in its effect. The initial thickening of the hypodermis might be supposed to be due to some other agent, perhaps another hormone. Prevention of wing development would occur only in individuals in which the inhibiting hormone reached certain concentrations (Fig. 1, B). High concentrations would thus result in winglessness, not in wings as in A. Intermediates could again be produced by moderate concentrations or a spread in the period of determination.

It is not necessary to assume that the hormone will increase in amount; it may diminish. A concentration sufficient to cause wing-bud thickening may be present in all embryos, but in some individuals under certain conditions become diluted later so that further development is not stimulated (Fig. 1, C).

These examples will suffice to illustrate the supposed action of the hormone. Other schemes are possible.

#### ALTERED RATES OF HORMONE PRODUCTION AS SOURCE OF INTERMEDIATES

It has generally been assumed, on any scheme like the foregoing, that intermediates owe their existence to modified rates of production of the stimulating or inhibiting hormone. Any rate which brings the concentration to some level between the thresholds of beginning and of complete development, at or before the critical time of determination, should lead to intermediacy. On this hypothesis, the several postulated schemes of hormone action illustrated in Fig. 1 should produce results having one thing in common, namely, a relation of frequency of intermediates to frequency of winged individuals. The reasons are briefly the following.

If embryos start development as of one morphological type, and if under given conditions many but not nearly all of them finish as of another type, numerous embryos must have crossed the threshold or thresholds separating the two types in development. The histology of prenatal hypodermis in aphids suggests that the development is at

first always toward the winged condition, but the argument may be made wholly general. If all the aphids are of one type in the adult stage, all of them may have crossed the thresholds, or none of them may have crossed. Conditions producing such a result are not very illuminating. When, however, the end results include both types in considerable numbers, many individuals must have crossed the developmental thresholds no matter what scheme of hormone control is in operation.

In the crossing of these thresholds lies the opportunity to produce intermediates, according to the theory involving rate of hormone production. The more individuals which have crossed them, the more intermediates there should be, other things being equal. If crossing the thresholds stimulates wings, the frequency of intermediates should be positively correlated with that of winged aphids. If crossing the thresholds inhibits wings, the frequency of intermediates should be negatively correlated with that of winged aphids.

#### PREVIOUS INDICATIONS OF CORRELATION OF INTER-MEDIATE WITH WINGED APHIDS

In certain groups of earlier experiments with the strain of aphids collected in Ann Arbor in 1923, it appeared that "in general the greatest production of intermediate wings does not accompany the greatest production of wings" (Shull, 1937). In those experiments "the correlation between the two is found to be  $r = -.68 \pm .10$ "; that is, "the fewer the wings the more numerous the intermediate wings." In another strain of aphids, however, "the greatest number of intermediate aphids would seem to be produced under circumstances which favored medium wing production. . . ."

The conclusions just quoted, which were not the primary goal of the previous studies, were vitiated somewhat because only total numbers of winged and of intermediate aphids in each family were used. An appearance of negative correlation might be created by a very strong genuine

negative correlation in one part of the family together with no correlation or mildly positive correlation in another part. Such a condition would scarcely indicate a real connection between the two classes of aphids.

#### DISTRIBUTION OF INTERMEDIATES IN THE FAMILY

The distribution of both intermediate and winged offspring in the family has now been ascertained for aphids reared in environmental conditions which produce enough intermediates to make a reliable comparison. Only the 1923 strain of aphids was used because the 1931 strain produces too few intermediates in almost all conditions. The distribution was ascertained by transferring the parents to a new plant every two days (if at 24°) or every five days (if at 14°). The offspring produced on each plant were reared as a group and the number of winged and intermediate-winged among them determined. Usually from six to eight successive plants were required to include practically the entire family.

The results of these tests are presented in Table 1. The letters *a*, *b*, *c*, etc., designate the successive plants, hence the successive groups of offspring. All aphids produced on plant *a* of the several families are combined into one total, all those on plant *b* of the several families into another total, and so on. The total number of aphids of all kinds (including the gamic forms which were uncommon) is given for each plant, and the winged and intermediate-winged ones are given as percentages of these totals. If it is desired to know the actual numbers of individuals, these must be calculated from the total and percentages. Too much attention should not be paid to the last groups of offspring in most experiments, for the total numbers of aphids are so small that percentages may well rise or fall in meaningless ways.

The percentages of winged and of intermediate-winged offspring in this table are graphically shown in Fig. 2. Within each rectangle are shown the winged and intermediate aphids in the experiments carried on in one set of

TABLE 1

SHOWING THE DISTRIBUTION OF THE WINGED AND INTERMEDIATE OFFSPRING IN THE FAMILIES OF DIFFERENT KINDS OF PARENTS REARED AT VARIOUS CURRENT AND AMBIENT TEMPERATURES. ALL PARENTS WERE REARED IN CONJUGAL LIGHT DURING THE EXPERIMENT. THE COLUMNS HEADED a, b, c, ETC., INCLUDE SUCCESSIVE GROUPS OF OFFSPRING FROM THE BEGINNING OF THE FAMILY TO THE END

Type of parent	Temperature at which			Offspring on successive plants									
	Parents' ancestors	Parents were reared to 4th instar	Parents were kept from 4th instar on, and offspring were born	Plants		a	b	c	d	e	f	g	h
				Types of data	of data								
Wingless	24°	24°	24°	Total	1967	1158	1160	836	505		142	5	
				% Winged	55.52	23.58	13.02	18.90	25.74	21.83	0.00		
				% Int.-wd.	1.63	3.38	2.67	2.51	5.74	20.42	20.00		
		24°	14°	Total	1314	1291	1091	967	786		142	1	
				% Winged	42.92	3.49	4.22	12.62	18.96	2.11	0.00		
				% Int.-wd.	3.12	2.25	3.94	6.83	12.60	7.75	0.00		
		24°	24°	Total	1528	1091	942	760	701	218			
				% Winged	56.28	37.53	36.73	48.55	53.78	55.05			
				% Int.-wd.	2.16	3.76	5.10	9.61	9.13	11.47			
	14°	14°	14°	Total	890	1046	1105	1010	922	310	23		
				% Winged	58.97	1.72	19.10	18.45	18.88	28.98	0.00		
				% Int.-wd.	3.71	7.17	11.13	12.67	10.95	4.52	4.35		
Alt.	Alt.	24°	Total	1844	1081	977	904	576	165				
			% Winged	60.63	30.53	14.84	26.44	41.84	49.09				
			% Int.-wd.	1.52	2.96	2.56	2.54	5.21	15.15				
		14°	Total	1219	1146	1199	1007	798	807	26			
			% Winged	41.67	3.66	5.17	7.45	6.64	0.00	3.85			
			% Int.-wd.	3.77	2.27	9.76	12.51	14.66	5.21	3.85			
Alt.	Room	24°	Total	827	589	569	386	216	104	18			
			% Winged	62.88	19.69	14.41	21.24	44.44	55.77	22.22			
			% Int.-wd.	1.21	2.21	2.28	4.40	6.02	12.50	16.67			
Alt.	Room	14°	Total	555	585	538	523	328	184	47			
			% Winged	60.36	3.25	2.04	11.85	14.94	8.70	0.00			
			% Int.-wd.	2.70	1.37	5.02	9.94	25.61	25.00	4.26			
Room	Room	24°	Total	1321	881	687	530	343	193	4			
			% Winged	70.25	36.66	15.87	16.04	27.70	43.52	25.00			
			% Int.-wd.	0.38	4.99	3.06	2.45	6.12	5.69	0.00			
		14°	Total	657	717	604	438	389	220	23			
			% Winged	52.66	2.37	1.16	2.97	3.34	13.18	0.00			
			% Int.-wd.	3.65	2.79	2.48	4.34	14.40	17.73	13.04			

TABLE 1.—(Continued)

Type of parent	Temperature at which			Offspring on successive plants									
	Parents' ancestors were reared	Parents were reared to 4th instar	Parents were kept from 4th instar on, and offspring were born	Plants		a	b	c	d	e	f	g	h
	Types of data												
Winged	24°	24°	24°	Total	2029	1398	1372	1013	679	244	42		
				% Winged	37.41	35.55	26.02	42.45	69.81	48.77	71.43		
				% Int.-wd.	2.51	4.58	3.35	4.64	3.89	9.84	0.00		
	14°		14°	Total	3144	3804	3780	3164	2706	527	219		
				% Winged	26.27	12.57	21.11	20.04	18.07	12.33	19.18		
				% Int.-wd.	2.77	4.23	8.10	8.69	8.06	8.16	5.02		
	14°	14°	24°	Total	2999	2019	1153	411	268	140	37		
				% Winged	16.11	26.94	29.84	33.58	45.15	51.43	27.03		
				% Int.-wd.	1.00	1.58	3.90	9.49	15.30	6.43	21.60		
	14°		14°	Total	1058	997	839	651	380	137	22		
				% Winged	14.46	11.43	10.25	14.13	6.32	0.00	0.00		
				% Int.-wd.	2.36	8.24	15.26	24.88	18.16	10.95	4.55		
	14°		24°	Total	894	743	591	425	332	234	101		5
				% Winged	40.83	22.61	16.07	19.76	30.12	48.29	61.39		100.00
				% Int.-wd.	1.68	1.48	3.05	1.18	3.61	7.26	6.93		0.00
	14°	Room		Total	544	735	577	550	354	335	74		
			14°	% Winged	29.23	4.76	7.28	7.45	6.50	7.76	2.70		
				% Int.-wd.	2.39	2.86	10.23	13.82	21.47	9.85	13.51		
Winged	Alt.		24°	Total	2677	1885	1489	1401	1072	584	102		37
				% Winged	41.73	29.92	36.20	50.82	66.04	56.85	63.73		70.27
				% Int.-wd.	2.09	3.71	4.78	5.35	5.50	8.39	3.92		8.11
	Alt.	Alt.	14°	Total	1619	1881	1626	1636	1474	960	45		40
				% Winged	23.04	8.40	13.47	10.64	12.08	7.12	2.22		10.00
				% Int.-wd.	3.03	7.18	13.53	16.32	14.52	13.64	20.00		15.00
	Alt.		24°	Total	938	716	664	475	327	293	98		34
				% Winged	21.75	13.83	20.78	21.26	32.72	57.68	48.98		94.12
				% Int.-wd.	0.11	0.98	2.71	2.11	6.73	3.41	3.06		2.94
	Room	Room	14°	Total	548	611	624	517	442	331	89		53
				% Winged	10.40	2.78	5.77	11.03	8.14	7.55	8.99		0.00
				% Int.-wd.	1.64	2.13	6.25	14.31	13.80	18.13	13.48		0.00
	Room	Room	24°	Total	2082	1352	1343	1013	799	394	32		
				% Winged	42.65	25.59	17.80	23.69	38.30	56.09	31.25		
				% Int.-wd.	3.03	2.51	2.98	4.74	7.51	13.20	6.25		
	Room	Room	14°	Total	1285	1422	1494	1300	1158	571	34		
				% Winged	28.33	3.94	7.50	8.23	4.75	7.18	2.94		
				% Int.-wd.	2.57	2.81	7.90	16.92	18.22	12.43	2.94		
?	?	14°	Total	2057	2579	2790	2177	1981	556	312			
			% Winged	28.25	13.88	17.24	18.19	13.58	14.93	14.74			
			% Int.-wd.	3.31	3.84	5.56	6.11	5.40	6.29	5.13			



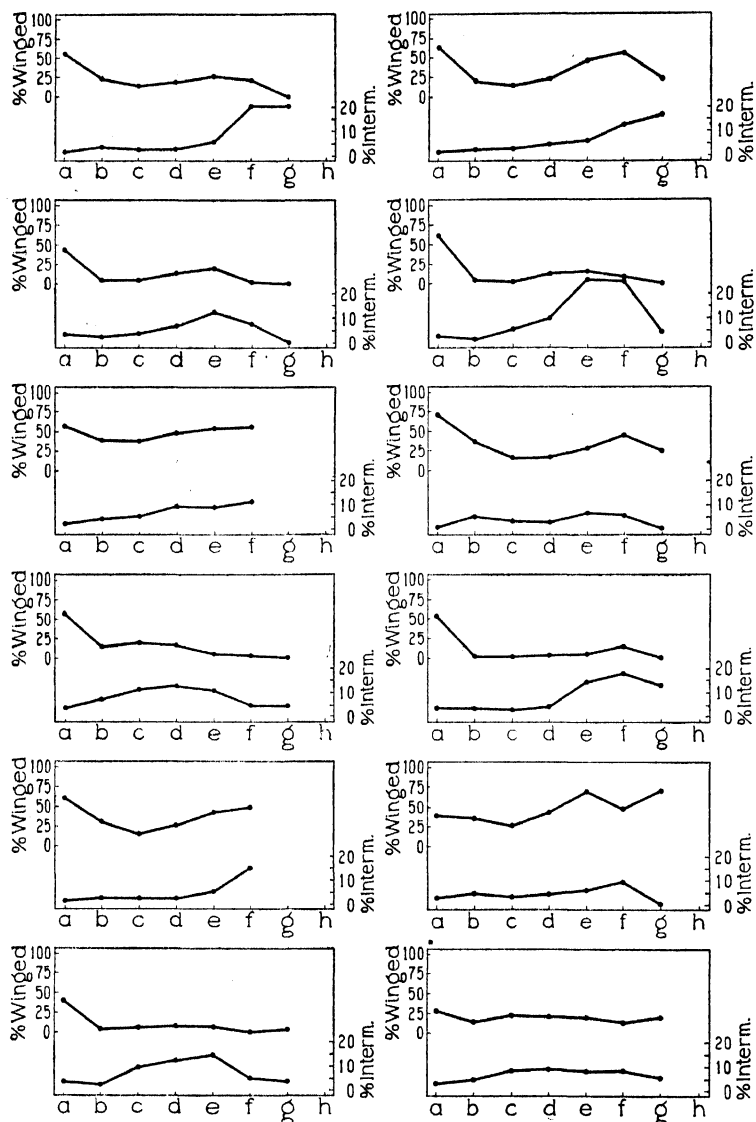


FIG. 2. Curves representing the percentages of winged and intermediates in the successive groups of offspring, based on the data of Table 1. Each rectangle represents one of the 23 groups of parental characters and treatments in that table.

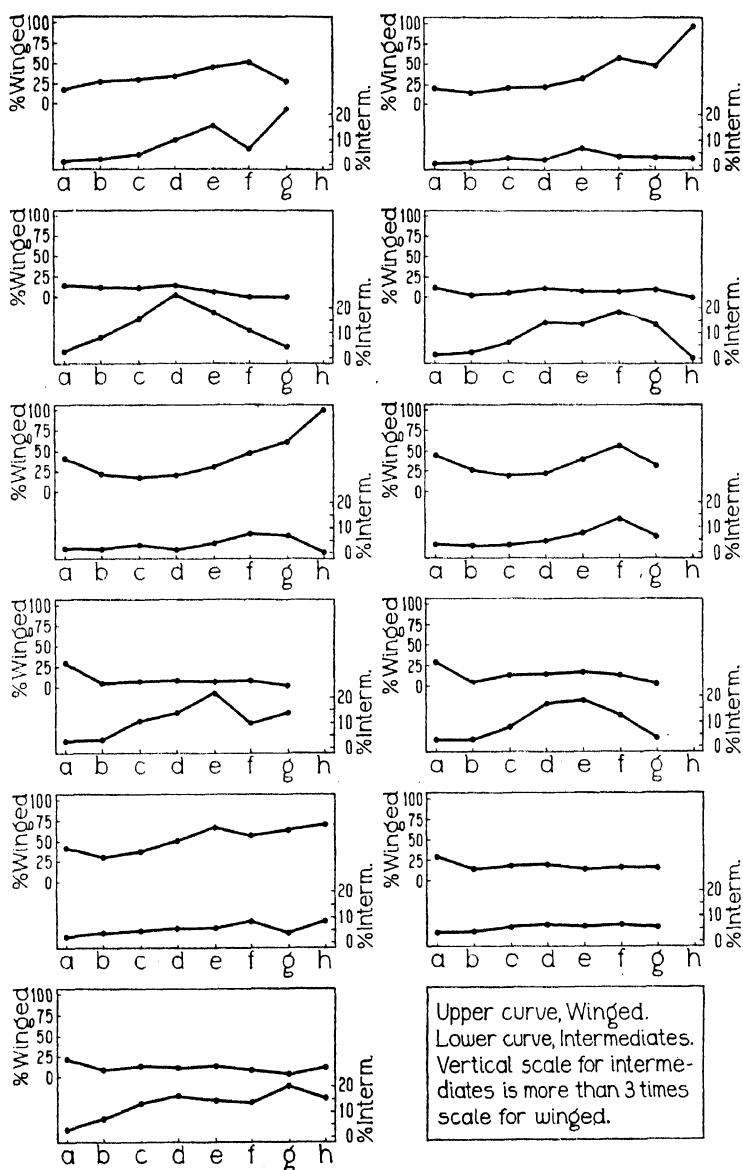


FIG. 2 (Continued).

conditions with parents of a given nature and origin. The 23 rectangles thus refer to the 23 trios of lines in Table 1. The upper curve in each represents the winged offspring, the lower curve the intermediates. The curve of intermediates is drawn to a larger scale than that of the winged, in order to discriminate more accurately between the generally lower percentages of intermediates. No confusion is likely to result from this difference in scale.

#### RELATION OF WINGED APHIDS TO INTERMEDIATES

The curves show a very strong tendency for the winged offspring to be numerous at the beginning of the family. In most of them the percentage of winged offspring on the first plant is never again equaled on any succeeding plant. Even where the later *percentages* are greater than the first one, they are usually based on total numbers so much smaller that the actual numbers of winged aphids late in the family is smaller than the early ones. When, therefore, total numbers of the two kinds in the family were alone considered, as in the former study (Shull, 1937), the percentages were largely determined by the percentages early in the family. Since the early groups of offspring included on the average many winged ones and few intermediates, a negative relation (somewhat less striking) was imposed upon the totals.

This negative relation is not clearly indicated by the later groups of offspring. Sometimes there is an opposite trend of the two curves, as in the first rectangle where, toward the end of the family, the percentage of winged offspring declines while that of intermediates rises. A similar opposition in greater detail is observable in the last two groups of offspring in the eleventh rectangle. Sometimes the two curves are similar (after the first lot of offspring), as in the second pair of curves and less strikingly in the 8th and 10th pairs. More often, however, the crests and troughs of one curve appear to be placed quite irrespective of the crests and troughs of the other.

There is one possible weakness in the present data, in

that the offspring borne on each plant were usually derived from several parents. At the outset 6 or 8 parents were put on the plant; these gradually died until the last plant often bore only one or two. It is conceivable that the intermediate-winged individuals were produced by one or two of the parents, and the winged offspring by the other parents; or the winged and intermediate offspring by certain parents, wingless ones by other parents, and so on. Such a condition would mean, however, that each aphid was more or less a law unto itself in its developmental response to light and temperature. While the scheme of developmental control has been proved to be very flexible with respect to the differentiating characters of gamic and parthenogenetic females (Shull, 1933), with numerous modifications of it in individuals, nothing so erratic has been found as would be involved in the assumption that individual differences would create the appearance of correlation where none existed, or destroy the signs of correlation where it actually occurred. The experiments would have to be conducted in a much more laborious manner to eliminate any conceivable error from this source. In the meantime it is assumed that the results mean what they appear to mean.

#### ANOTHER POSSIBLE SOURCE OF FREQUENCY OF INTERMEDIATES

Since the frequency of intermediates is not clearly a function of the frequency of winged aphids, other relations must be sought. It will be observed from the curves that the peak of production of intermediates, if there is a peak, occurs late in the family. In two of the curves it is about the middle, in all the others in the latter half. In no group are there more intermediates in the early part of the family than at any later time.

These facts indicate some dependence of intermediates upon age. To all appearances it is the age of the mother that thus governs frequency of intermediates among her offspring. It would be possible, however, to regard the

aging as occurring in the protoplasm of which the offspring are composed, since oöcytes must be nearly contemporaneous in their origins and some of them are long delayed in their conversion into embryos. It is impossible to decide at present where the age effect occurs.

The nature of this age influence can only be conjectured. Certain probabilities may be assumed, however, regarding the mechanism through which it modifies the frequency of intermediates. The absence of a clear connection between numbers of intermediates and numbers of winged or wingless indicates that this mechanism does not involve a change in the rate of production of the stimulating hormone such that the curve of concentration of the hormone crosses the thresholds of wing production or wing inhibition either more or less frequently.

The remaining sources of more intermediates, which are in harmony with the prevailing concept of developmental control, are a spread between the concentration which will initiate wing development and that which will insure its completion, and an extension of the period of determination. Either of these would suffice, but both could operate simultaneously.

The spread of concentrations of the hormone would appear to have the larger range open to it, and is accordingly favored. Such a spread should be at least as liable to modification by age as would length of a period of determination. It is possible, also, though hardly probable, that the age effect is in some degree comparable to the influence that arose in an old parthenogenetic line after six years of laboratory culture (Shull, 1932), when it reversed some of its differential responses and rendered them at the same time less definite. The validity of this comparison would depend on whether age effects could pass over from one generation to the next through the egg or through some cumulative maternal influence on the embryos; and it is impaired by the fact that the old parthenogenetic line changed the frequency of its production of intermediates rather suddenly instead of gradually.

The speed with which old lines change their responses is under investigation in another connection at present.

#### SUMMARY

Previous work indicated that, as judged from the total number in the family, there is a negative correlation between the frequencies of winged and intermediate-winged aphids. This could be interpreted to mean that intermediates result chiefly from changed rates of production of a stimulative (or inhibiting) hormone. When the distribution of intermediates and of winged aphids in the family was studied, however, it was found that no such regular relation existed. The apparent correlation arose from a strong tendency to produce winged offspring at the beginning of the family, at which time the intermediates were always few. Intermediates are in some degree an accompaniment of age. As such, they are more probably dependent on a wider spread between the thresholds of beginning and complete development of wings, or on a lengthened period of determination of wings, in older parents, rather than on changed rates of hormone production. The spread between thresholds is favored.

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# COMMENSALISM AND DOMESTICATION<sup>1</sup>

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## I

COMMENSALISM is a close partnership on equal terms between two individuals or groups of individuals. The partners do not influence each other, but simply live together under the same general conditions of environment. This forms a contrast to conditions obtaining under symbiosis on the one hand and parasitism on the other. Symbiosis, like commensalism, is a partnership on equal terms. But the partners influence one another profoundly, and are not able to exist without each other. Parasitism, on the contrary, is an unequal partnership. The parasite itself is not able to exist outside the host; he lives off the host and is injurious to him.

The commensals which it is proposed to discuss in this connection are all mammals, and they are all commensals of man. They include a mouse, 4 species of rats and a shrew. It has been suggested that these animals are not really commensals, but that they are parasites. But it is quite obvious that from the biological point of view it does not matter whether they sit at man's table or whether they live off his table. The whole idea, it seems to me, is adapted from human conditions, and the difference suggested may have an economic or a social background, but it has no biological meaning at all. As the main features pointed out to be characteristic of the commensal, namely, loose partnership under the same conditions of environment with man, apply to these animals, there is no reason to regard them as anything but commensals.

## II

I shall now give a short description of the species we are going to deal with.

<sup>1</sup> Lecture, Biological Laboratory, Harvard University, January 21, 1938.

The first of them is the house-mouse, *Mus musculus* L. This is now a cosmopolitan animal. Its origin, ancestry, evolution and history are well known and will be discussed in detail later on.

The second species is the house or black rat, *Rattus rattus* L. The origin and the ancestral type of this animal are known in a general way, but in a general way only. What can be said with certainty is that the house-rat has been developed from a species of rat found wild in the Malay area; but we do not know yet the subspecies, nor have we been able to form any idea whether one or more than one subspecies are ancestral to the commensal, that is, whether the commensal is monophyletic or polyphyletic. We also may regard it as a proved fact that the actual differentiation of the commensal has been effected in continental India and that migration has begun there. From India the house-rat has reached the Mediterranean and Europe over land, on the trail of Neolithic man, and its world-wide distribution has had its center in Europe. This animal is largely transported by shipping across the ocean and along inland waterways, like the Congo or Amazon. Its routes of migration can now be largely reconstructed.

The lesser Malay house-rat, *Rattus concolor* Blyth, is our third type. This species is not cosmopolitan. It is restricted to the Malay area and Oceania as far as Hawaii and New Zealand, where it is the common house-rat. Its origin must be Malayan, although a connection between the commensal and the wild stock has not been established. In fact, we are not quite certain what the wild stock is like.

Another member of the genus *Rattus* has become as important and ubiquitous a commensal as the house-rat; indeed, being the stronger of the two, has replaced it in many places. This is the brown, or Norway rat, *Rattus norvegicus* Berkenhout. The actual wild ancestor, as in the lesser Malay rat, remains to be established. But the species as such can be traced back as far as southeastern China, from where it has spread to the north and west



and has reached Europe on the northern transcontinental route. From there, like the mouse and the black rat, it has spread elsewhere.

The East African Negro rat, *Mastomys coucha* Smith, it not very widely known. It is found all over Africa, and it has a tendency to live in Negro huts. Like other commensals in close connection with man it has developed identical changes, chiefly in color. Although its distribution is limited, it has become important, as, like the house-rat, it carries plague.

The last commensal to be dealt with, the large Indian musk shrew, *Suncus caeruleus* Kerr, is not a rodent but an insectivore. The fact that this is a commensal is not generally known, indeed, the connection between the wild stock of continental India and the various commensal types has not been worked out at all. Most of them have been described as separate species or subspecies, and that was all. But there can not be any doubt that we have to deal with a true commensal. We know that it lives in houses and native house-boats, and that it has been carried by man. Its present distribution, in addition to continental India, includes the whole of Malaya and southern China, and it is also found all along the east coast of Africa and the shores of the Red Sea.

### III

The question may be asked why all these animals are regarded as commensals, and not as wild animals which have looked for shelter and food in houses and other human settlements, and run wild again if and when they want to. This is true of early commensal types, but it is not so in more advanced commensals which have developed changes under commensal conditions that make them fitted for an indoor but less fitted for an outdoor life.

### IV

The changes produced by commensal life are various. They affect the type of color, and they control the pigment itself. They affect the general size of the body, but also

that of its various parts, the length of ears, of feet, of the tail. They are evident in the shape of the skull, in particular one change, the reduction of the length of the face compared with that of the brain case. These changes may be transitory, non-inheritable, and may disappear in the offspring. But the true commensal characters are real mutations. Why and how they appear we shall see later on.

## V

The commensal best known at present is the house-mouse. During the last two years I have myself had an opportunity to do some research on this species. Almost everything is now known of its ancestry, evolution and history. It is, therefore, that I shall try to point out the conditions that lead to commensalism, and to explain the changes that are typical in the development of commensal characters, on the evidence of the results obtained in the study of this animal.

The house-mouse group as it now exists is not a homogeneous crowd. There are three wild ancestral stocks representative of three different subspecies of the species *Mus musculus*. All the house-mice of East Central Europe and of European and Asiatic Russia have sprung from a wild stock of their own, and the same is true of the Japanese house-mouse. But all other house-mice in the world, whatever the local type may be, can be traced back to one and the same root. This wild stock is the Central Asiatic *Mus musculus wagneri* Eversmann, which is found all over the dry area of Central Asia from the Pacific, through northern China, Mongolia, Turkestan, and as far west as the Volga River. It has been possible definitely to prove that the development of the commensal out of the wild stock began in northern Persia, and that migration and the evolution of commensal types has taken place both in an eastern and western direction. Two different groups have been formed which are identical in most of the more typical commensal characters, although these have been developed independently of one another, only under the same conditions of habitat.

The eastern or Indian series inhabits Iran, India, Malaya, southern China and Australia; it has been carried into Oceania, and is known as far east as the Marquesas Islands. It is also the common type of house-mouse in East and South Africa, where it has been brought by shipping. The western, or European, series is found in Mesopotamia, northern Africa and western Europe. From there it has reached the Atlantic islands and has become a typical inhabitant of the American continent, being found both in the northern and southern hemispheres.

Let us now consider a few of the changes that can be observed in the evolution of the commensal mouse which distinguish it from the wild stock and which may be taken as typical commensal characters. I shall pick out (1) the concentration of the yellow pigment; (2) the pigment control; (3) the length of the tail; (4) the shape of the face and its relative length compared with that of the brain case.

In the Indian series four different kinds of yellow can be distinguished; they are buff, ochraceous, tawny and cinnamon. The palest shade occurs in the wild stock, ochraceous is found in the form *bactrianus*, which occurs in Iran and northwestern India. Tawny is typical of the Punjab commensal *tytleri* and of the Himalayan forms *homourus* and *urbanus*; the darkest stage, cinnamon, is found as an alternative in the same two forms. In short, the whole progress may be described as an intensification of the yellow tint which passes from buff to a shade which is rather reddish brown than yellow. All these stages represent mutations which make up an allelomorphic series of a variable factor which is designated as the concentration of yellow.

A similar series of mutations occurs in the factor that controls the distribution of pigment in general, and which is responsible for the demarcation between the color of the back and that of the belly. This series is well known to geneticists as the Agouti series. In the wild stock, *Mus musculus wagneri*, the belly is perfectly white, with

a sharp line of demarcation along the flanks, with the tail bicolored, and the hands and feet white and sharply set off. In the Punjab *tytleri* we find a buffy belly and buffy feet, with the line of demarcation slightly obliterated both along the flanks and on the tail. This type of coloration is also characteristic of the south European *Mus musculus brevisrostris*. The next step in the series represents an animal with a gray belly and drab or sepia feet, without any line of demarcation along the flanks, and with a uniformly colored tail. This stage is represented by some of the Indian commensals, like *M. m. urbanus* and *M. m. manei*, and by the typical European *M. m. musculus*. The last stage of this series, all black, is not known anywhere in the mouse as a type forming whole populations, but it must be mentioned here because it occurs in the house-rat and the African Negro rat, and because we shall have to deal with it in connection with domestication.

As regards the length of the tail the wild stock has it much shorter or at most just as long as the head and body. But with advancing commensalism the tail may, and does, increase in length. True commensals may have the tail just slightly shorter than the body, but as a rule have it much longer. The genetic analysis of this factor is only in the beginning; but it appears that stages in tail length can be traced, and that they make up a series of alleles corresponding to the one studied by Madame Dobrovolskaia-Zavadskaia (1934) and in Professor Dunn's Laboratory in New York (Chesley and Dunn, 1935, 1936).

The changes of the skull that take place in the evolution of the house-mouse have been described by Gates (1926) in connection with his researches on the Japanese waltzing type. But they hold good for all commensals and serve to distinguish them from all the wild stocks. The chief character exhibited by the commensal is the shape of the facial portion, which is shorter than in the wild animal, with the rostrum more slender, compressed laterally and from above. How far these changes are inheritable, and in which way, nobody knows for certain, as the prob-

lem has not yet been touched by the genetic experiment. But they clearly represent a juvenile condition and may be due to some change in the endocrinal balance, which is controlled by unknown genetic factors.

It should be pointed out here that these mutations are not restricted to the mouse, but that they appear again in all the other commensal species.

## VI

If we examine more closely these various series of mutations one fact becomes evident. All the mutations which appear in commensals and develop in a series are recessive mutations. This is perfectly clear and well established in the Agouti series. But from Nachtsheim's experiences (1929) in the rabbit the same applies for the concentration factor for yellow where the dominant mutations are pale, and the recessive mutations are intense. As far as we know at present, long tail is recessive to short tail, and again we have the recessive stage in the commensal and the dominant end of the series in the wild lot.

The question will be asked, are these recessive mutations produced by commensal conditions of life and why do they not appear in the wild stock. The reply is: they do appear in the wild stock, but being less resistant to wild conditions, they are either discarded because they have a reduced selective value or they look for shelter and become commensal. With increasing recessiveness commensalism becomes more strongly pronounced.

On the other hand, it is a fact that dominant mutations occur in the commensal as they do in the wild stock. Some of them occur in both<sup>2</sup> and do not seem to have any particular selective value under the conditions given. But from what we can ascertain from the actual commensal populations, it would appear that those alleles which are dominant over the local commensal type are not retained under commensal conditions. They either have a nega-

<sup>2</sup> One of these is the mutation "sharp dorsal zone," i.e., a dark back sharply defined along the sides of the body; this corresponds to the "Verdunkelungsfaktor" of German authors.

tive selective value and disappear, or they run wild and become feral. Wheresoever we have two types of the same allelomorphic series in one area, the stage representing the recessive allele remains indoors, while the dominant type is found in the open.

## VII

If recessive mutation proceeds, a stage will soon be reached where the disestablishment of the gene destroys all chance of surviving. Indeed, the lower stages of the albino series are entirely unable to exist even under commensal conditions. They have a reduced ability to produce immunity against infections, and exhibit all sorts of physiological disabilities.<sup>3</sup>

But quite a few of the more recessive mutations are able to exist in captivity or in a state of domestication. Domestication is but an exaggerated condition of what we find in the commensal stage, and in fact primitive commensals are not really different in their make-up from the commensal. They show, for instance, the same increase in yellow pigment, the same deficiency in pigment control, and the same type of juvenile skull which we have traced in the commensal mouse.

But in addition to that and increasing with more highly developed domestic conditions, we meet with more advanced stages of recessive mutation, such as the development of deep chestnut and brown out of the paler tints of the yellow series in the horse, cow, pig, but also whites and piebalds which in the wild stage, or even as commensals, would be impossible, except under very special conditions. This state of things is possible on account of the high degree of protection given by man to the domestic animal,

<sup>3</sup> See also Kleinschmidt (1936). The objection may be made that recessive mutations, even of the albino series, are able to survive in a wild state. Acromelanism, corresponding to Himalaya white, is not uncommon in birds, as for instance the tyrannid *Xolmis irupero* Vieillot or the fruit-pigeon *Ducula bicolor* Scopoli. But it appears that in these cases the recessive mutation fits into a balanced set of genes, whereas in the commensals under consideration the genetic balance has been upset under conditions of reduced control by natural selection.

but also to the process of artificial selection which preserves the dominant mutations, and thus counterbalances the evil effects of recessiveness in certain genes. Without this artificial process domestication would lead to degeneration and extinction. The partnership between man and the domestic animal, therefore, is an unequal one, the benefit, in a biological sense, being entirely on the side of the domestic animal.

### VIII

This account would not be complete without a consideration of man himself, as he is the other partner of the commensal animal, and as he lives under the same general external conditions of environment. Has he the same tendency to produce recessive mutation? It would appear that in more than one character he follows the road of the commensal. Compared with primitive man, modern human types have a shortened face. Moreover, they have a tendency to become lighter and to lose the black pigment. From the limited amount of genetic data that we have from man, but more so from the data we have from Primates,<sup>4</sup> it can be taken as a well-supported theory that this development proceeds in a recessive direction. But even more than in the domestic animal this weakening of his constitution may be regarded as balanced, and overbalanced, by the protection man is enabled to provide for himself through his own intellect.

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<sup>4</sup> Schwarz (1934).

## SHORTER ARTICLES AND DISCUSSION

### A REINVESTIGATION OF THE SO-CALLED SEX CHROMOSOMES IN *MELANDRIUM* (*LYCHNIS*) *ALBUM*

*Melandrium* (*Lychnis*) *album* has become one of the classic examples of sex determination dependent upon the behavior of a pair of unequal chromosomes during the reduction division. A reinvestigation of this angiosperm was undertaken to compare the results obtained from studies on the genus *Rumex*. In the dioecious members of the latter group various sex-determining mechanisms have been described among the chromosomes the nature of which involves an association of three bodies which segregate to the daughter nuclei of the pollen mother cells, adding two chromosomes to the one complement and one to the other. The behavior of this mechanism as described by numerous authors was found to be highly irregular. Many associated phenomena of the reduction divisions suggested a hybrid origin for these unisexual species. The same peculiarities ascribed to the sex chromosomes in the unisexual species were also found in the natural hybrids of some of the hermaphroditic species. Consequently, the author could hardly credit the peculiar history of certain chromosomes in the dioecious species as having a primary influence on the sex of any resulting generation.

With this in mind, the behavior of the so-called sex chromosomes in *Melandrium album* proves interesting. As in the case of *Rumex*, a superficial study might persuade one that the apparatus as described is without reproach. In this instance the sex chromosomes are supposed to form an unequal pair in the male during meiosis. So they do; but not always. The exceptions, most often found during the early anaphase, distinctly show that what might often appear to be an unequal pair is basically an equal pair, each member of the pair being in turn made up of two distinct pieces joined end to end. As in *Rumex*, where the supposedly tripartite chromosome revealed a tetrapartite nature, this supposedly unequal pair in *M. album* upon close study shows itself to be an association of two pairs of chromosomes. What makes them look like an unequal pair is due to a difference in the attachment and form subsequently taken by the halves of the



association. Often the end piece on one side is bent under the more equatorial piece so that a shortening seems to have occurred. It also appears that during the process of separation one of the equatorial members procures some of the chromatin from the other equatorial piece. Of course this adds to the inequality. During the second meiotic division the polar view of the metaphase plate often reveals that the so-called sex chromosome varies in form, depending presumably upon how much chromatin it either lost or gained in the first meiotic division.

A glance at the mature pollen sacs suggests that *M. album* too is of hybrid origin in that a great deal of imperfect pollen and occasional microcytes are present. The degree of imperfection varies with the anther sac; some being almost devoid of any perfect grains, while others possess largely perfect pollen.

In the author's opinion the validity of sex chromosomes in *M. album* is to be treated with skepticism because of two principal arguments. In the first place, the apparatus, when closely studied with improved technique, displays similarities to certain conditions in known hybrids and thus seems more closely associated with abnormalities induced by previous hybridization than with the direct determination of sex. It must also be pointed out that as one reviews the list of Angiosperms for which sex chromosomes have been claimed one is impressed with the fact that these very species belong to groups some of whose members are hermaphroditic and that they usually belong to fairly large genera in which natural hybridization is a common occurrence. Though research on the species in question has not been completed, there seems to be adequate reason to doubt the existence of a clear case for the presence of sex chromosomes in *M. album*.

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#### THE LONGEVITY OF INSECTS DURING COMPLETE INANITION

My attention has been called to the fact that, in a recent paper (1938), I have set at odds, as it were, two prominent students of insect bionomics. The passage under concern (p. 41) ran thus: "... the mature larvae and adults of the North American grasshopper, *Chortophaga viridifasciata*, endure [approximately] the same length of time (5.5 to 6.6 days at all relative

humidities, including 0 per cent. R. H. Ludwig (1935) therefore suggested that the cause of death is starvation rather than desiccation. One would, however, suspect that the death of these grasshoppers was caused by a factor of which Ludwig was not aware, since Bodine (1921) had found that these grasshoppers "will live well beyond a hundred days without the intake of solids or water." Bodine, however, had stated "hours" and not "days." The results of the above investigators are thus in accord.

There exists a rather prevalent notion that insects are capable of marked resistance to complete starvation. This die-hard bias was undoubtedly the factor which caused me unintentionally to substitute "days" for "hours" when abstracting Bodine's paper. The following table, containing data collected at random, shows that most adult *active* insects are *incapable* of surviving more than a few to several days during complete starvation at ordinary temperatures and atmospheric conditions. Thus the active domestic fly (Glaser, 1923) can live for approximately 1.5 days without any food and water but will live about a month when normally nourished. A similar relationship holds for *Drosophila*.

There are, however, notable "exceptions." Thus, 8-mm larvae of the beetle, *T. tarsale*, can endure inanition for at least 6 years (see table). At the end of that time the larvae had become reduced to the size at hatching. Dufour (1933) kept an adult *Cimex lectularius* (bed-bug) in a closed container without food and water for a whole year (April, 1826–April, 1827), at the end of which time it was still vigorous. Kemper (1930) has recently made numerous extensive studies on the longevity of this bed-bug under various conditions and found a mean of 140 days for the longevity of the adults during inanition; and this only after the animals had completed a repast of mammalian blood (*cf.* table). The wife of a Belgian official in the Congo told me, several years ago, of a certain poisonous beetle in that country which is believed to be capable of enduring 8 years in complete starvation. I would like to learn, from scientific sources, more about this beetle.

The larvae of insects which undergo an extensive metamorphosis, *i.e.*, of insects which have a relatively pronounced fat-body, can endure starvation for a longer period than the adults of the same species. This is probably because of the large nutritive *dépôt* and higher water percentage of the larvae. The main source of energy, during the starvation of insects, is fat

TABLE 1

Species (adults unless otherwise specified)	Approx. mean time (in days) for death during complete abstinence from food and water	Temp. ° C.	Investigators
<b>ORTHOPTERA</b>			
<i>Melanoplus femur-rubrum</i> . . . . .	2	22-25	J. H. Bodine (1907)
<i>M. differentialis</i> . . . . .	3	"	"
<i>Chortophaga viridifasciata</i> (hibernating larvae) . . . . .	7	23	"
<i>C. viridifasciata</i> (larvae and adults) . . . . .	6	25	D. Ludwig (1935)
<i>Periplaneta orientalis</i> . . . . .	20-60	Room	E. W. Sanford (1918)
<b>NEUROPTERA</b>			
<i>Hemerobius</i> sp. . . . .	2	"	H. J. Lucas (1926)
<i>Perla bicaudata</i> . . . . .	2.5	"	"
<b>HEMIPTERA</b>			
<i>Trialeurodes vaporariorum</i> . . . . .	42	22	H. Weber (1931)
<i>Cimex lectularius</i> (bed-bug) Stage I larva (from completion of meal) . . . . .	70	22	H. Kemper (1930)
Stage I larva (sobered) . . . . .	35	"	"
Stages II and III (from completion of meal) . . . . .	112	"	"
Stage V (from completion of meal) . . . . .	133	"	"
Young males and females (from completion of meal) . . . . .	140	"	"
<i>C. lectularius</i> (adult) . . . . .	365 (at least)	Room	L. Dufour (1833)
<b>ODONATA</b>			
<i>Libella cancellata</i> . . . . .	3	"	B. Slowtsoff (1905)
<b>COLEOPTERA</b>			
<i>Chrysomela populi</i> . . . . .	6	"	F. L. A. W. Sorg
<i>Dermestes lardarius</i> . . . . .	13	"	"
<i>Cerambyx fuliginator</i> . . . . .	36	"	" cf. Lucas (1926)
<i>Lampyris noctiluca</i> . . . . .	2	"	Fingerhuth, unpublished (cf. Lucas 1926)
<i>Coccinella 14-guttata</i> . . . . .		"	Fingerhuth, unpublished (cf. Lucas 1926)
<i>Calandra granaria</i> . . . . .	3	"	Fingerhuth, unpublished (cf. Lucas 1926)
<i>Curculio scrophularia</i> . . . . .	5	"	Fingerhuth, unpublished (cf. Lucas 1926)
<i>Melolontha horticola</i> . . . . .	6	"	Fingerhuth, unpublished (cf. Lucas 1926)
<i>M. vulgaris</i> . . . . .	21	"	Slowtsoff (1904)
<i>Geotrupes stercoralis</i> . . . . .	8	"	Slowtsoff (1909)
<i>Geotrupes</i> sp. . . . .	14	"	Fingerhuth
<i>Lucanus cervus</i> . . . . .	27	"	"
<i>Cetonia aurata</i> . . . . .	30	"	"
<i>Tenebrio molitor</i> (larva) . . . . .	ca. 240	27	H. Sing-Pruthi (1925)
<i>T. molitor</i> . . . . .	30	Room	G. Teissier (1928)
<i>Trogoderma tarsale</i> (museum pest) . . . . .			
(1-mm. larva) . . . . .	ca. 240	"	J. E. Wodsdalek (1917, 1921)
(8-mm. larva) . . . . .	2,190 (at least)		
<b>LEPIDOPTERA</b>			
<i>Bombyx cerura</i> (does not take food normally) . . . . .	15	Room (June)	Lucas
<i>B. mori</i> (does not take food normally) . . . . .	3	" "	"
<i>B. mori</i> (larva) . . . . .	8	" "	V. L. Kellogg and R. G. Bell (1904)
<i>Deilephila euphorbiae</i> . . . . .	12	" "	K. Farkas (1903)
Sphinx moth . . . . .	12	" "	J. Heller (1926)

TABLE 1—(Continued)

Species (adults unless otherwise specified)	Approx. mean time (in days) for death during complete abstinence from food and water	Temp. ° C.	Investigators
<b>HYMENOPTERA</b>			
<i>Formica fusca</i> .....	2	" (May)	Lucas
<i>Apis terrestris</i> .....	3	"	"
<i>A. mellifica</i> .....	5	" (June)	"
<i>Vespa vulgaris</i> .....	8	"	"
<i>Bombus terrestris</i> .....	1.5	"	Slowtzoff (1905a)
<b>DIPTERA</b>			
<i>Ophyra cadaverina</i> .....	6	"	F. Tangl (1909)
<i>Musca domestica</i> .....	1.3 (without water)	" "	S. I. Vinokuroff <i>cf.</i> Pearl and Parker (1924)
	1.8 (with water)		
	2		
<i>M. domestica</i> .....			R. W. Glaser (1923)
<i>Drosophila melanogaster</i> (wild and vestigial-winged) .....	2 (both sexes; ♀ somewhat longer than ♂♂)	25	{ R. Pearl and S. L. Parker (1924), and W. M. Barrows (1907)
<i>D. ampelophila</i> .....	2 (same remark as above)	25	F. E. Lutz (1915)

(Slowtzoff, 1904, 1905, 1909, 1905a, Tangl, 1909, Kopeć, 1924, Heller, 1926), and Slowtzoff believed that in most such cases death ensues when the fatty stores have been largely consumed. At the time of death the percentage of dry substance lost is approximately constant. Teissier (1928) found this to be true regardless of the temperature (10°–37° C.).

Next to fat, under ordinary atmospheric conditions, water is the substance lost to a most marked extent (expressed in per cent. of the initial quantity of water). Since the bumble-bee and dragon-fly die after losing only a relatively small fraction of their fats, Slowtzoff believes that water-loss is a prominent factor in the short duration of these insects during starvation, even though they do not lose more water than several other species which live longer. In accordance, a great deal of evidence can be presented to show that insects vary considerably in their tolerance to water-loss. In the instance of *C. viridifasciata* (*vide supra*) water-loss is apparently not the limiting factor. In fact, the water *percentage* of this animal rises when starved at an R. H. of 96 per cent. (Ludwig, 1935); but this does not, however, imply that a loss in the total water *content* does not occur. In most cases (including other grasshoppers, studied by Bodine, 1921) it is clear that water-loss is, other things being equal, a limiting factor, since longevity during starvation is considerably prolonged if water is provided (see, for instance, Bodine, 1921, Vinokuroff, see table).

On the whole, it appears that the duration of life during complete starvation is, to a certain extent, a function of the normal life span and this again is, to a certain degree, a function of the metabolic rate. Such a generalization is not amenable to rigid application on its sole merits. Thus, while the normal life span of the wild type *Drosophila melanogaster* (Pearl and Parker, 1924) is about three times that of the vestigial-winged type, the duration of both types during inanition is equal. I have plotted data collected at random in an attempt to exhibit a supposed relationship between the normal metabolic rate (oxygen consumption rate) at a given temperature (20°–25° C.) and the duration of life during inanition of species exemplifying approximately the entire animal kingdom and find no general relationship between such; this is true even when the plottings are restricted to a single class—the insects.

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## FURTHER STUDIES ON INCUBATION OF TURTLE (*MALACLEMYS CENTRATA* LAT.) EGGS<sup>1</sup>

IN earlier reports (Cunningham and Hurwitz, 1936, and Cunningham and Huene, 1938) it was shown that the increase in weight of various reptile eggs during incubation could be accounted for, at least for the most part, by the absorption of water.

The studies did not positively exclude the possibility that mineral salts were also taken up by the egg. An examination of the data of Karashima (1929) indicates that both mineral salts and carbohydrates increase in the first fifteen-day period of incubation in the albumen and yolk of the sea turtle, *Thalassochelys corticata*. The source of this increase was not specified, although it may be assumed to be the shell. The present studies consider the whole egg as a unit and attempt to discover whether minerals from the environment enter the egg.

<sup>1</sup> These experiments were carried out at the U. S. Fisheries Laboratory at Beaufort, N. C., and the authors express appreciation to the bureau for the use of its facilities, and to Dr. H. F. Prytherch, director, and Mr. Charles Hatsel, foreman, for their cooperation.

It is unfortunate that the complete story can not be followed in each egg and that a somewhat statistical method has to be followed in order to ascertain whether or not elements other than water are added from the environment during incubation. Such procedure involves the use of a reasonable number of eggs at each of the selected ages. In these experiments the number was limited by the time available for the analyses. The eggs were studied individually, since by this method significant variations might become evident.

Newly laid eggs were taken from the nest before it was covered. These were weighed and immediately desiccated and reweighed to a constant figure. The dried mass was then ashed and weighed. Other eggs were incubated for 33 days under normal conditions and a similar procedure followed. Still others were incubated for 60 days and similar data collected.

The composite data for each group are shown in Table I. The data show a very slight increase in dry weight in the 33-day group over the fresh eggs. This would appear, however, to be well within experimental error. The increase in ash weight, however, is greater, but when taken into consideration with the slight rise in organic materials it is probably again within the range of experimental error.

TABLE I  
WEIGHTS OF ASH AND ORGANIC MATERIAL AT VARIOUS STAGES OF DEVELOPMENT

No. eggs	Days incubated	Average wet wt.	Max. Min.	Average dry wt.	Max. Min.	Average org.* wt.	Max. Min.	Average ash wt.	Max. Min.
20	0	10.581	11.068	3.117	3.445	2.764	3.072	.3531	.4341
			7.283		1.829		1.574		.2478
			14.826		3.672		3.356		.4313
20	33	12.441	10.701	3.155	2.769	2.784	2.529	.3714	.3120
			16.585		3.645		3.090		.4160
			11.273		2.005		1.727		.2597
25	60	13.326		2.800		2.429		.3712	

\* Organic material.

The variations in dry weight, ash content and organic material between eggs in a single group are far greater than the differences in averages of the different groups. While there is much overlapping from one group to the other the maximum ash in each

group shows a steady but comparatively slight regression during incubation.

Taken as a whole, it appears as if the mineral salts (ash) are reasonably constant during incubation. Further evidence for this conclusion, secured by a different procedure, will be presented later. The slight rise in organic material, when there should have been an appreciable loss at 33 days, can not be so easily explained, but the considerable drop before the 60th day indicates it may be an error in technique; it might be accounted for either by proportionately thicker shells or larger yolks in this particular group of eggs.

The problem of absorption of minerals was also approached from another angle. Eggs were placed upon a rack in a humidity chamber. A piece of cheese-cloth, one end of which dipped into distilled water, was placed under the eggs and folded back so as to cover the tops of the eggs. A glass plate, slightly raised to allow admission of air without too rapid evaporation of water, was placed over the top of the chamber. The distilled water was changed from time to time to prevent stagnation. A control, using tap

TABLE II

COMPARISON OF NEWLY HATCHED TURTLES FROM EGGS SUPPLIED WITH DISTILLED WATER ONLY WITH THOSE INCUBATED IN NATURE

	Num- ber em- bryos	Aver- age wet wt.	Max. Min.	Aver- age dry wt.	Max. Min.	Aver- age organ. wt.	Max. Min.	Aver- age ash wt.	Max. Min.
Normal	10	7.860	9.8141	2.138	2.9056	1.915	2.636	.223	2.693
			7.2409		1.9710		1.754		2.054
			7.8594		2.2412		1.976		2.485
Experi- mental	5	7.517	7.2613	2.055	1.9382	1.830	1.721	.225	2.107

water (artesian well), was also set up. The jars were exposed to ordinary room temperature. In both cases the development proceeded to hatching, and gross examination revealed no anomalies of development, either in form or time required for hatching as compared to the normal in nature. The newly hatched embryos, supplied during incubation with distilled water, when analyzed and compared with embryos hatched under normal conditions, showed no significant differences. The data are given in Table II.



It is quite evident that all the mineral salts necessary for development are contained in the eggs; in fact, there is an excess, which may be partly accounted for by the minerals remaining in the shell at the time of hatching.

These experiments indicate that when the egg of the diamond back terrapin is taken as a whole it contains (with the exception of water) all the ingredients, in sufficient quantity, to insure the proper development of the embryo.

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## CHROMOSOME STRUCTURE<sup>1</sup> ON COILING IN CHROMOSOMES<sup>2</sup>

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THE present paper is an attempt to construct a mechanical model of the coiling of chromonemata. As a speculation standing between the observational facts obtained with the aid of the microscope and the existing knowledge of biochemistry of chromosomes the present image is to serve the biochemist as well as the microscopist as a reference point for further approaches.

There exist with regard to the mechanism of coiling two main schools of speculation which may be termed the matrical and the molecular. Exponents of the former are Huskins, Kuwada, Sax and others; of the latter, Darlington.

Wilson and Huskins (1939) write:

As the simplest possibility, let us consider the space-limiting factor to be a pellicle<sup>3</sup> which remains nearly stationary in size while the chromonema is elongating. Let us further assume, in accord with morphological evidence (apart from functional considerations), that this pellicle forms prior to the beginning of spiralization and in some manner disappears prior to the unravelling of the spiral in first pollen-grain division prophase.

<sup>1</sup> Presented at the joint symposium of the American Society of Zoologists and the Genetics Society of America in conjunction with the American Association for the Advancement of Science at Richmond, Virginia, December 28, 1938.

<sup>2</sup> Approved by the Director of the N. Y. State Agricultural Experiment Station as Journal Paper 307. Jan. 6, 1939.

<sup>3</sup> Darlington, who, contrary to us, doubts the existence of a "matrix," agrees (1936b) that: "There are several grounds—chiefly non-morphological—for assuming that the chromosome thread has some sort of pellicle."

Some of the expected properties of the spiral which this method of formation would produce are:

(1) If elongation is gradual and uniform throughout the length of the chromonema and coiling is not caused by an internal torsion, an irregular zig-zag would be expected in the early stages of spiralization.

(2) Any point of interruption of the spiral such as chiasmata, the attachment region, bends in the pellicle, or any lack of homogeneity in the chromonema and its matrix, might cause changes in the direction of coiling (with random frequency).

(3) Chromatids, closely associated during spiralization, should coil in the same direction.

(4) Uncoiling would be expected to be gradual.

Kuwada (1937) writes:

The mechanism of chromonema coiling we assume to occur in two steps.

(1) The chromonema threads are brought to a twisted state by intensification of the internal tendency by anisotropic swelling. (2) When imbibition water is distributed evenly the threads are forced to untwist. The untwisting is realized by transformation of the twist into a spiral, the matrix playing a great rôle in this transformation and also bringing the spiral into the regular form. When the contracting force of the matrix is lost spirals become loose. The internal twisting (1) and the matrical force (2) which contracts are regarded as two inseparable factors.

Kuwada also introduces the plant *tendrils* to illustrate reversals of direction of coiling which compensate for twists in one direction by corresponding ones in the other.

Sax and Humphrey (1935) state:

... coiling can be simulated by compressing two closely associated flexible wires in a glass tube while the ends of the wires are not permitted to rotate. The chromatids are undoubtedly somewhat elastic and flexible as shown by their behavior at division. They may be prevented from rotating within the pellicle by the association between the fiber attachment points and the pellicle. Inhibition of rotation at the distal ends of the chromosomes may also be affected to some extent by the terminal or subterminal chiasmata.

(This discussion pertains to meiosis primarily.) Thus the chromonema has elastic properties and is passively helicated. The matrix is responsible for contraction and spiralling.

In the scheme of Kuwada and in the now abandoned scheme of Huskins and Smith anisotropic growth is responsible for coiling. Sax does not suggest what determines the pitch and the regularity of the spiral.

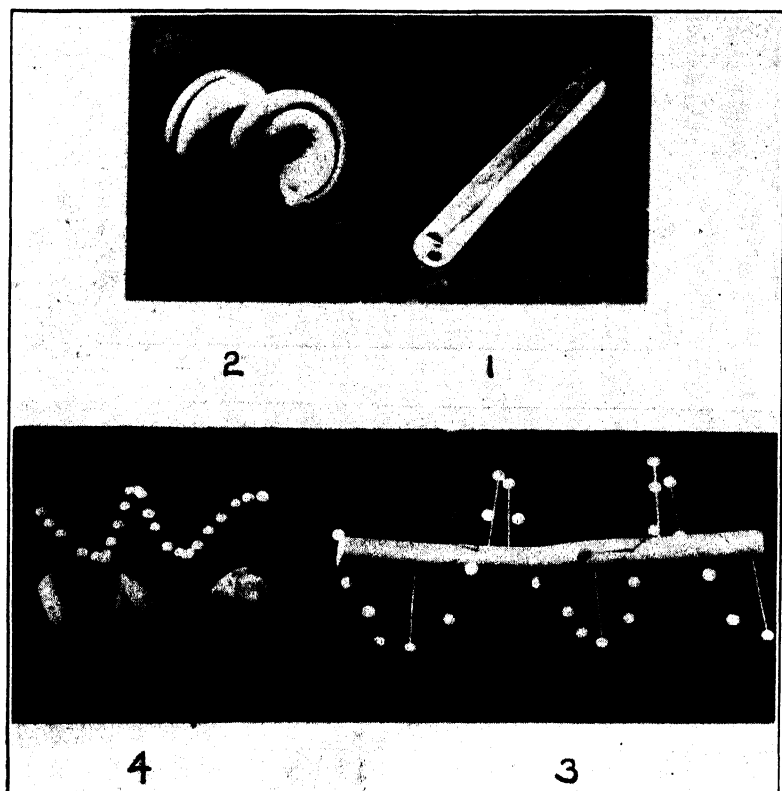
In contrast to all the foregoing Darlington (1935) links the reason for coiling to some basic asymmetry of the

molecules constituting the gene thread. This is implied in his term *molecular coil*. He states that the spiral (coiling of chromatids or helication) arises by an internal twisting of the thread in opposite direction to the spiral assumed, and that since the molecular spiral must determine both major and minor spirals, these must be in the same direction and opposite to the twist that conditions or determines them. One may thus contrast the "matrical" and the "molecular" schemes of coiling, and the essential features of these schemes are illustrated in two pairs of models, Figs. 1, 2 and 3 and 4.

It would be difficult to construct a model of the entire chromosome including matrix, chromonema and gene string. Hence the matrical space has been omitted in the models of Figs. 1 to 4 but may be imagined by loosely enclosing, in a mental picture, each model in a Cellophane bag. In Fig. 1 is shown a cylindrical body, which stands for the chromonema proper. Its axis constitutes the genonema. The model carries a cleat running along its entire length; this stands for a heterogenic sector of the chromonema and is attached to the outside of the cylinder to make it more obvious. Chromonemata in the microscope being cylindrical, such a sector if existing would not project from the periphery of the chromonema. Fig. 2 shows the same model as Fig. 1, but the chromonema is now helicated. This process may have come about by expansion of the cleat, which would at first tend to spiralize the thread, but being confined in the matrical bag a helix will result.

In this model the cleat may be omitted. If the matrix is rigid and the chromonema elongates within it and if the latter is thought of as having physical properties of a comparable rubber rod, a coil will result. This is the picture of Wilson and Huskins (1939). Unfortunately not all investigators agree that the thread is straight and relatively short during prophase and elongates toward metaphase.

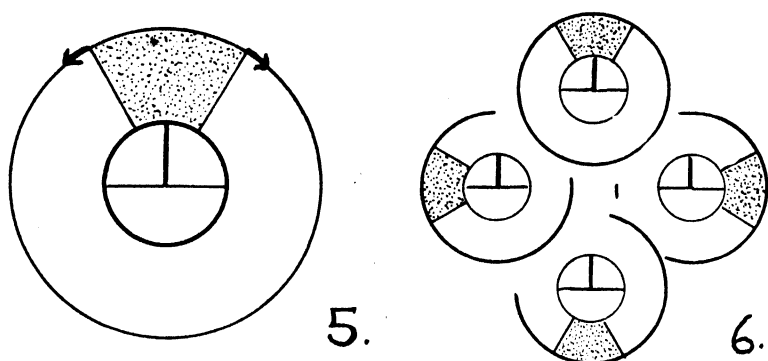
In such a structure as discussed above the coiling of the straight thread into a helix would result in a twist of the thread. If, on the other hand, one wishes to maintain ulti-



Figs. 1 and 2. Models of matrical concept. Clay models of coiling as occasioned by sectorial (heterogonic) swelling. Fig. 1 is a straight thread (cylinder) with heterogonic sector (cleat) which upon swelling will cause coiling (Fig. 2) if the structure is confined in a limited cylindrical space.

Figs. 3 and 4. Models of the nemameric concept. Clay models of nemameric (molecular) coiling. In Fig. 3 pins are arranged in a helix around a central thread. The pins represent points of contact for external forces with individual thread units (nemameres). Fig. 4 shows how an external force oriented in space (polarized) will pull successive pinheads in one general direction and will cause the thread to coil in the opposite direction of the nemameric twist. The action of the polarizing force in the model is assumed to have started at one end.

mate units of the straight thread in the identical orientation in space in spite of coiling, the heterogonic sector (the cleat of the model in Figs. 1 and 2) will have to rotate around the core. This is illustrated in Figs. 5 and 6, which are diagrammatic cross-sections of a chromonema of the



Figs. 5 and 6. Models of matrical concept. Diagrammatic cross-sections through a genonema as described in Figs. 1 and 2. Fig. 5 is the cross-section of the straight thread. Fig. 6 shows 4 successive cross-sections of one gyre of the helix in which the heterogonic sector must migrate around the center if the latter remains untwisted in space. Heterogonic sector dotted. Arrows indicate alternative directions of migration.

type illustrated in the models of Figs. 1 and 2. The cleat or heterogonic sector is shown as a dotted area and the arrows in Fig. 5 indicate that this sector may migrate around the center in either one of two directions. Fig. 6 gives four consecutive cross-sections of the chromonema, taken at distances one quarter of a turn of the helix apart from one another. It may be seen that the shaded area is always on the outside of the central figure. One may ask why it is necessary to assume that the coiled gene string maintain its orthotropic (untwisted) orientation in space: If coiled threads can multiply in the coiled condition and separate freely without uncoiling, *cf.* ring-chromosomes, multiplication and hence orientation would have to be orthotropic for all units of a given thread. According to Huskins (1935) and according to Kuwada (1937), the gene string is twisted in the relaxed state, in the coiled state it is untwisted. Speaking teleologically the chromonemata coil so that daughter chromonemata may separate without entanglement. It is thus tacitly assumed or implied by Huskins and by Kuwada that the gene strings at the time of multiplication (interphase) may be wrapped around one another. In Kuwada's scheme it is not stated clearly what determines the twisted sector in the relaxed thread.

Darlington (1935) introduced the molecular spiral. One assumes that consecutive molecules of the gene string show some systemic asymmetry which will make the thread "twisted." This principle and the superstructure of speculation suggested by Darlington have been generally rejected primarily because he fails to suggest a mechanical principle which will explain randomness of direction of coiling and random reversals of direction of coiling.

In the following I intend to resurrect the principle of the molecular coil but to endow it with properties which will make the direction of coiling facultatively determined anew at each period of gene multiplication. Certain changes in terminology suggest themselves which evolve from the existing uncertainty about orders of magnitude and mechanical qualities of molecular aggregates.

"Molecular spiral" implies that the basic units of the gene string are single molecules and these are asymmetrically built. I would like to think of the *genonema* as being built up of units which may be aggregates and call the basic unit of the gene string a *nemamere*. The *nemamere* which may be a fibril would stand in size between the gene and the *chromomere*, the latter being a visible unit, while the former, in ordinary light, is not.

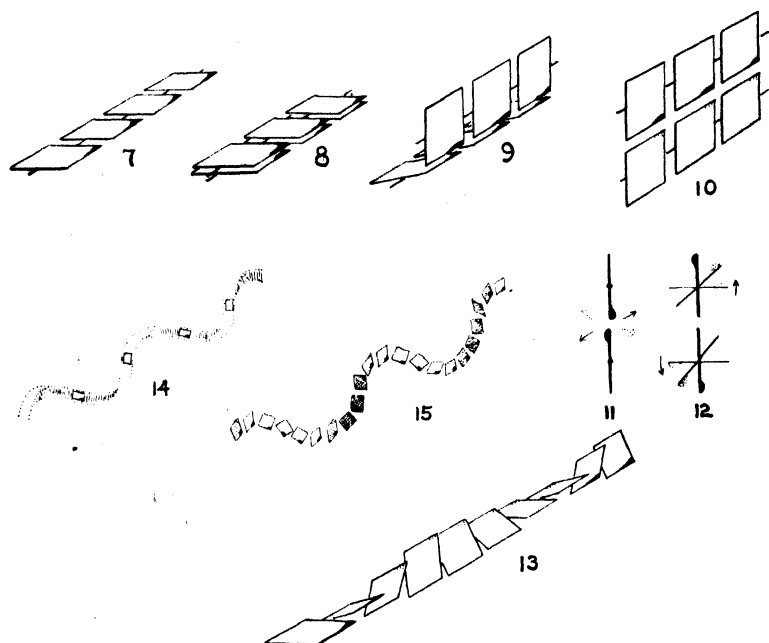
Figs. 3 and 4 illustrate the principle of the *nemameric helix* (molecular spiral) which coils the thread by untwisting. The clay bar is the main axis of the thread which is made up of individual units; in this model each unit consists of a pin and a piece of the clay bar. The individual units are joined semi-rigidly. The orientation shown in Fig. 3 is called the *nemameric twist* since each unit is twisted in space relative to the next one. A helix is formed by the axial thread if the pins are forced into a parallel orientation. Thus a coil "results" from the twist. If the *nemameric twist* is *sinistorse* the resultant coil will be *dextrorse* and *vice versa*. In the model the series of pinheads stand for contact points on which directional forces may act. This model represents my interpretation of Darlington's (1935) description.

In the "relaxed state" (Darlington) the chromonema of this model is straight in space, but, as shown in the model, twisted with regard to the pinheads projecting from it. According to my own interpretation a thread in this condition is not completely relaxed. Further relaxation might undo also the spiral of pinheads by separating individual unit parts enough so as to overcome the effect of their individual geometric asymmetry. Any orthotropic attractive force starting from a given point and acting on the pinheads will tend to throw the thread into a "reverse" spiral. As is seen from Fig. 4 the pinheads thus come much closer together in space. If there is any repulsion between them and yet oriented attraction from the outside the thread will lengthen while it is coiling. This actually occurs in Trillium, according to Wilson and Huskins (1939). It may be noted from Fig. 4 that the pinheads form an orderly helix which is detached and completely separated in space from the original thread around which the pinheads previously (Fig. 3) formed a helical track. Hence daughter threads, if wrapped around one another when first formed, would become free of each other through their combined helication. What might be expected of the unit parts of a genonema of the general type illustrated in Figs. 3 and 4 and how can one obtain random coiling in a model of this kind?

This is my modification of Darlington's principle:

In the diagrams 7 to 13, nemameres are pictured as flat rectangles with a projection called x-point in one corner. This x-point will, when nemameres are aligned closely (primary contraction), allow successive units rotational end-to-end freedom around the main axis of the genonema only through, *e.g.*, 320 degrees of the arc. In the completely relaxed state nemameres are so far apart that they form a flat band (Fig. 7). Nemameres multiply by reproducing themselves in space according to Figs. 8, 9 and 10. They are pictured as unfolding as the leaves of a book. The x-points cause the respective edges which carry them to break apart last. At this time there comes into play





Figs. 7-15. Models of the nemameric concept. Fig. 7 shows a piece of a genonema made up of flat nemameres carrying in one corner an x-region which serves as a point of contact with external forces. The genonema is now extended or relaxed and not coiled. Each nemamere has a leading (away from the observer) and a trailing edge, an x-point edge and a neutral edge. In Fig. 8 each nemamere has multiplied, forming an identical replica of itself in space below or above itself. In Fig. 9, daughter genonemata separate by unfolding, the x-point edges of the nemameres acting as hinges on a door or as the back of a book on two leaves. Fig. 10 shows daughter nemameres ready to separate but still held in position by attraction of x-point edges. Nemameres with backs toward the observer are shown with dotted x-points. In Figs. 11 and 12, leading and trailing edges of nemameres are shown in projection. The thickening indicates the position of the x-points. As genonemata separate mutual attraction of sister x-points gives way to mutual repulsion, which causes sister nemameres to rotate away from one another in one of two opposite directions. Solid arrows signify sinistrorse, dotted arrows dextrorse mutual repulsion. The reaction spreads from one unit to the next, piling successive nemameres "upon" one another, as indicated in Fig. 12 for three successive nemameres. Leading edges, due to their x-points will now repel the next trailing edge enough to prevent it from coming to perfect alignment. Fig. 13. As a result of the reaction shown in Figs. 11 and 12 daughter genonemata will show an isodirectional nemameric twist. Pairs of sister genonemata will be twisted dextrorsely or sinistrorsely. The twisted genonema shown in Fig. 13 is a result of shortening of the axis of the genonema or an expansion of the nemameres. The thread is now no

rotational repulsion between daughter x-point edges, and this will result in these edges revolving away from one another in one of two directions. Either direction may be chosen at random by the first pair of nemameres separating from one another. The projection of this movement is shown in diagram 11, in which the line stands for the leading and trailing edge of the nemamere the thickening for the x-point. Such a reaction is believed to start from certain points and spread from there to adjacent nemameres as the reaction in a chain might spread from one link to the next. Diagram 12 shows the result of such an event. At the same time when daughter nemameres separate, primary contraction within threads may occur. Successive nemameres will be stacked upon one another if the x-points now function to give each thread the final structure shown in Fig. 13, in which we now recognize the molecular spiral or in present terminology the nemameric twist. The nemameric twist of Fig. 13 may be transformed into a coil by expansion of the nemameres and intussusception or heterogonic growth of the x-point edges or by further (secondary) contraction. Thus a coil would be formed in which the gene string would be twisted (Fig. 14). There is, however, another alternative.

By exertion of an external polarizing force acting to

longer relaxed; in Fig. 13 it shows a sinistrorse nemameric twist. Dotted x-points indicate the reverse side. Fig. 14. The genonema shown in Fig. 13 will form a helix as a tape wound around a pencil as a result of further expansion of the nemameres without the interference of external forces. This helix will be in the same direction as the nemameric twist, sinistrorse in the case of Figs. 13 and 14. This is one alternative of coiling. The other is described in Fig. 15. Fig. 15. A dextrorse spiral will result from the condition shown in Fig. 13 if an external force unwinds the nemameric spiral and orients all x-points toward one main direction in space. The peculiar arrangement of individual nemameres is due to the resultant of two forces acting on each of them—A, the external orienting force, pulling all x-points towards a plane below the helix, and B, the repulsive force between x-points and the following (trailing) edge of the next nemamere. This latter force causes all x-points to be further removed from the observer than the next trailing edge. Trailing edges or at least their lower ends show a relative right-shift away from the x-points. The resultant of these two forces on the genonema as a whole is in our case a dextrorse helix.

draw all x-point edges into one direction in space a nemameric twist would be transformed into a helix, thereby undoing the nemameric twist. This may or may not be accompanied by further (secondary) contraction of the thread. If the polarizing force is active immediately after the separating and rotational mechanisms come into play, the torque of the latter will be counteracted by the untwisting effect of the former just a few nemameres up the line and the genonema will split, twist and untwist and hence coil in scarcely separated events of a compound occurrence. Sister threads will coil in the same direction and hence separate readily. Gene multiplication could conceivably take place at any stage of the cycle, but nemameric separation is significantly linked to the beginning of a new coil. Likewise reversals of direction of coiling in a given piece of a gene string can only take place concomitantly with nemameric reproduction. In certain organisms straightening of the gene string may be very incomplete (*Tradescantia*), in others it may be complete. The scheme may be applied to meiosis as well as to mitosis. It likewise holds if nemameres produce their own mirror images instead of identical units. In this case genes and nemameres would be paired units at all times.

Contraction of the genonema may occur as follows: Completely relaxed, the thread is straight and there is no nemameric twist. Primary contraction at the time when daughter threads separate helps to establish the nemameric twist. Secondary contraction may enter in when the chromonema coils. Both primary and secondary contraction may be substituted for by varying the reactive forces of the x-points, allowing of chromonema elongation during prophase, in case cogent evidence to this effect should accumulate.

What in the above scheme is new, what is old, and what are the advantages of the scheme? The principle of the molecular coil, as suggested by Darlington, has been used to explain how basic units which are asymmetrically built may form a helix. The principle of the heterogonic sector

in a thread as suggested by Kuwada and Huskins and Smith has been used, inasmuch as one might look upon the x-points of the nemameres of the present scheme as projections of the heterogonic sector of the thread onto the units of its interior. The mode of separation of reduplicated genomemata and the randomness of direction of the nemameric twist as derived from the mode of reduplication as well as the integration of the principles mentioned are believed to be original. It appears desirable that further speculations on coiling may start from different premises and assumptions, since it is only too true that many assumptions now appearing justified may become obsolete in the near future.

*In summary:* It is speculatively suggested that the genomema is made up of nemameres represented by flat rectangular platelets carrying in one corner a characteristic called an x-point preventing rotational freedom over a small fraction of the arc. X-point edges separate last during nemamere reproduction. Torsional repulsion between daughter edges spreading from random points in both directions causes randomness of direction of the molecular coil now called nemameric twist. Helication occurs through orientation of subsequent x-point edges in one direction in space. The nemameric twist may thus be untwisted as fast as it is established.

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# THE PHYSICOCHEMICAL NATURE OF THE CHROMOSOME AND THE GENE<sup>1</sup>

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## 1. THE FIBROUS NATURE OF THE CHROMOSOME

METAPHASE and anaphase chromosomes usually appear as fairly thick solid rods. Suitable methods of fixation and staining (particularly pre-treatment with acids or ammonia vapor) reveal more structure. The chromosome consists of the chromonema thread, which in mitosis is coiled in a single tight spiral, while in meiosis this spiral may be coiled again in a second spiral. The mitotic spiral is known as the minor spiral and has typically about thirty turns. The spiral which is superposed on this in meiosis is the major spiral and has about five or six turns; it has so far only been described in plants.

Cytologists have not as yet by any means reached agreement as to the phenomena of coiling. The most important questions have been discussed by Nebel in another contribution to this symposium. The basic fact which remains perfectly clear is that, on the microscopic scale (i.e., structures with dimensions from a few hundreds to a few thousands of  $\mu$ ), the chromosome is built of a fine fiber. A similar conclusion may be reached from a consideration of the connections between the genes. As is well known, the genes are joined together in a linear array and must therefore have two ends which can join up with neighboring genes. There usually do not appear to be more than two such ends; if there were, we should be able to get branched threads in which one gene was joined to three others. Branched chromosomes have indeed been described, both on genetical and cytological evidence. But

<sup>1</sup> Presented at the joint symposium on "Chromosome Structure" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 28, 1938.

in some cases in *Drosophila*, Muller and Offermann have shown by investigation of the salivary glands that the genetical evidence was at fault, and the cytological evidence in other forms seems to require further confirmation. At present it seems justifiable to conclude that the gene has only two ends which are capable of stable attachment. This may not be true of the centromere, at which branching of the chromosome seems to be possible.

These two ends can not be regarded as poles. That is to say, the gene is not in any sense like a little magnet with different north and south ends. This is clearly shown by the fact that they can join up in reverse order in inversions.

## 2. THE SIZE OF THE GENE AND THE CHROMONEMA

Various attempts have been made to estimate the size of the gene. It is of course by no means clear that a gene has a definite and constant size; if the gene is a compound structure, it might grow gradually between two divisions. But the estimates of gene size are all rather rough at present, and it is very unlikely that the size of the gene is sufficiently variable to affect the order of magnitude. The estimates of size all aim at fixing maximum possible sizes; they show that, according to some particular line of reasoning, the gene or chromonema can not exceed certain dimensions. Clearly the most important datum will be the least maximum estimate.

All estimates of the dimensions of the gene start from considerations of the observed size of chromosomes or parts of chromosomes. Chromosomes certainly change in apparent dimensions during the processes of growth and division. Many of these variations can be explained as results of the changing degree of spiralization of the chromonema, and the relation between the gene and the chromosome, and thus the estimates of the size of the latter, always depends on the structure which we find or assume for the chromosome thread. The most securely founded of all statements about the condition of the chromonema is probably that it is completely straight and uncoiled in

salivary gland chromosomes. An estimate of the length of the chromonema associated with a gene can be made on this assumption. Muller and Prokofieva studied seven chromosome breaks occurring in a short region at the left end of the X chromosome in *D. melanogaster*. They found that the breaks apparently occurred in only four different places; and they concluded that these four places were the connections between five successive genes in the linear sequence. Further, they obtained a case of a minute deficiency which was viable in a homozygous condition and showed a phenotype indicating the absence of only two genes, yellow and achaete. By cytological examination of the salivary gland chromosomes in these cases they deduced the interval between successive genes to be about 125  $m\mu$ ; the yellow achaete deficiency was just visible as the loss of a part of a dark band.

Estimates of the widths of the genes can not be obtained directly from measurements of the widths of the chromosomes. In salivary glands, the individual chromonemata can not be distinguished, being presumably below the resolving power of the microscope. By ultra-violet light, at least 64 parallel threads can be seen in *Drosophila*, and up to 400 in the thicker chromosomes of *Chironomus*; there may be even more. In other stages, the apparent thickness is dependent partly on the spiralization and perhaps also on the amount of nucleic acid condensed on the thread. Muller has made an estimate of the maximum thickness of the chromonema and therefore presumably of the gene by assuming that when the chromosomes are most contracted at meiotic metaphase they are entirely filled with the coiled-up thread. The active part of the X in *D. melanogaster* has a volume of about  $1/12$  cu.  $\mu$ . The length of the thread, when uncoiled, is found to be about 200  $\mu$  in the salivary glands. If the thread has a square cross section with a side  $x$ , we can determine  $x$ , on the above assumptions, from the relation  $200 \cdot x^2 = 1/12$ , whence  $x = 20 m\mu$ .

This again is a maximum estimate, since there is no proof that the chromonema does fill the whole of the meta-

phase chromosome, part of which may be occupied by accessory material. Cases are known in which the dimensions of metaphase chromosomes are under genetic control; for example, in some species hybrids, chromosomes may appear up to ten times larger than they normally do, and this increase is almost certainly due to the accretion of extra chromatic material on to their surface. Moreover, it is probably that the inert regions of the chromosomes are not fully spiralized at metaphase, yet they appear, with most stains, to be of the same thickness as the active parts; this thickness must be due to the condensation of material on to the surface. There are then considerable grounds for supposing that a metaphase chromosome contains other material in addition to the chromonema, and that the estimate of the thickness of the chromonema given above is considerably too large.

Darlington has arrived at an estimate of volume by a method somewhat similar to Muller's; dividing the number of bands discernible in the IIIrd chromosome in the salivary glands of *Drosophila* (say 2,000) into the volume of the chromosome at mitotic metaphase, (say  $6 \times 10^7$  cu.  $\mu$ ) he finds the volume of the gene to be  $3 \times 10^4$  cu.  $\mu$ , which agrees fairly well with Muller's estimates given above.

The two best estimates we have obtained above are maximum estimates for the dimensions of parts of the chromonema; in the first place a length of about 125  $\mu$  seems to be associated with each gene, and secondly the maximum thickness of the thread is about 20  $\mu$ . If we can accept these two estimates, arrived at in different ways, as of about equal accuracy, it is perhaps significant that even the units of the chromonema are elongated, fiber-like bodies. But we must note that, quite apart from the possibility that these figures may overestimate the size of the chromonema-unit-particle, there is no proof that the whole of this particle is occupied by the gene. It is possible to assume that the gene is a very much more minute body embedded within and always associated with the larger particle whose size we have been estimating. We shall discuss this possibility further on.



### 3. THE CHEMICAL NATURE OF CHROMOSOMES

Chromosomes as such have never been chemically analyzed; they are too small for present methods. The nearest material which can be collected in quantities large enough for ordinary chemical investigation is sperm, particularly fish sperm. The head part of the sperm consists almost entirely of nuclear material, and analyses of this show that the two main constituents are thymonucleic acid (about 60 per cent.) and simple proteins of the kind known as protamines (35 per cent.). Nucleic acid combines very easily with proteins to form complex nucleoproteins, and it probably occurs in this combined form in the nucleus.

The distribution of nucleic acid in the nucleus can be investigated by means of ultra-violet spectroscopy, since it has a characteristic strong absorption at wave-lengths near 2,600 Å. During division stages, when the chromosomes are contracted and can be seen as separate bodies, almost all the nucleic acid is attached to the chromosomes; it may also be in the chromosomes in resting stages, but the fully extended chromonemata are not separately distinguishable from the nuclear sap, and the evidence is therefore not clear. The metaphase chromosomes can also be shown to contain protein, since they are attacked by proteolytic enzymes. In the salivary gland nuclei, the chromosomes contain the same two constituents, which perhaps makes it likely that the resting stage chromosomes are built up in the same way, and that the chromosome constitution is constant throughout the division cycle. Recently, however, Koltzoff has claimed that there is one stage in the development of the so-called "lampbrush" chromosomes in amphibian oocytes in which they contain no nucleic acid.

The chemical make-up of protein is not yet fully understood. It is known that some of the chemically rather inert proteins such as hair and silk are formed from fibrous elements consisting of chains of "polypeptide links," each link having the constitution-CO-CHR-NH-, where

R is a group (the side chain) which may be a simple hydrocarbon, an alcohol or a base such as arginine. In the fibrous proteins just mentioned, the links are arranged in linear chains, the chains being connected together by means of the side chains. There are, however, other types of proteins in which the molecules seem to be spherical rather than elongated; these are known as the globular proteins, and there are others intermediate between the fibrous and globular types. We do not know to which of these types the chromosome proteins belong, since the protein isolated from sperm (clupein) has not been examined from this point of view. The thread-like appearance of the extended chromosome, and the two-ended nature of the chromomeres, suggests that the chromosome consists of protein fibers arranged more or less parallel to its length. But this does not by any means necessitate the assumption that the chromosome protein is itself fibrous, since the orders of magnitude are quite different. The polypeptide links in a fibrous protein chain are about 0,35  $\mu$  long by 0,45  $\mu$  thick by 1  $\mu$  wide in the direction of the side chains. The visible chromonema is a few hundred  $\mu$  thick, while Muller's estimate, based on its length in salivary gland chromosomes, gives it a width of about 20  $\mu$ . Fibers as large as this can just as well be formed from globular as from strictly fibrous proteins, since the units (molecules or repeat cells in crystals) of the former are about 6  $\mu$  in diameter, and cases are known, for instance, in some of the virus proteins, in which these units unite to form fibers a few tens of  $\mu$  thick. Studies on the extensibility, and particularly the reversible extensibility, of the chromosomes give some, and could probably give much more, information about globular or fibrous nature of the chromosome proteins. In completely fibrous proteins the polypeptide chains lie fairly parallel and are more or less unfolded; they can only be stretched by actual straining of the chemical bonds. It is probable, however, that in globular proteins, the same or very similar polypeptide chains exist in a folded configuration, so that ex-

tension of fiber constructed of globular protein involves only the unfolding of the chains and can proceed much further before the fiber is ruptured. Duryee has shown that the lampbrush chromosomes of amphibian oocytes can be reversibly extended to about  $3\frac{1}{2}$  times their normal length, at least under favorable conditions (in absence of calcium or other heavy metallic ions). Salivary gland chromosomes easily stretch at least to twice their length and probably can be stretched further when special efforts are made to do so by microdissection methods. Thus even in chromosomes in which the chromosome thread or chromonema is apparently uncoiled, the thread itself has considerable elasticity, and perhaps may be constructed of globular proteins in which the polypeptide chains are folded on a molecular scale. Much further study is required, however, before this can be taken as more than a suggestion.

When we turn to consider the other main constituent of chromosomes, the nucleic acid, a series of facts emerge which are extremely suggestive of an essential connection between nucleic acid and proteins, but whose exact significance can not yet be stated. Nucleic acid itself easily forms fibers and x-ray studies have shown that these consist of a chain of phosphoric acid residues to the side of which are attached a series of flat, plate-shaped groups each of which contains a purine base attached to a sugar. The first remarkable fact is that the repeat distance along the chain, *i.e.*, the distance between neighboring phosphoric acid residues, is almost exactly the same as the repeat distance in a polypeptide chain; 0,336 for the nucleic acid, 0,334 m $\mu$  for the polypeptide. The difference, which may not be significant, is at least so small that it is easy to imagine that the polypeptide and nucleic acid chains might unite parallel to one another to give protein-nucleate chains. This can in fact actually be observed; Astbury has prepared the nucleate of clupein, the protein isolated from fish sperm, and shown that it is a fibrous material. Further confirmation comes from a study of the double re-

fraction. Protein fibers have a somewhat weak double refraction which is positive in the direction of the fiber, while nucleic acid, in which there are large flat plate-like groups sticking out at right angles to the length of the fibers, has a much stronger double refraction which is negative in the direction of the fiber axis. The clupein-nucleate shows a double refraction negative in the fiber direction due to the nucleic acid. So do fully uncoiled chromosomes, such as those of salivary glands and zygotene stages; when the chromonema is presumably coiled in a single coil (*e.g.*, mitotic metaphase) so that it runs perpendicularly to the length of the chromosome, the sign of the double refraction changes and becomes positive in the direction of the *chromosome* axis, while in meiotic metaphase, where the minor spiral is coiled again in a major spiral, the double refraction reverses again and becomes once more negative in the direction of the chromosome axis. All these data fit in very well with the idea that the protein and nucleic acid have combined to form composite fibers in which the two constituent fibers lie parallel to one another.

The cytological evidence makes it quite clear that the chromosomes are not homogeneous structures. In the first place, there is a differentiation in salivary gland chromosomes between the darkly stained bands and the non-stained inter-band regions. The property of stainability depends on the content of nucleic acid, and the concentration of this substance in the bands can be demonstrated directly by studies of ultra-violet absorption. One must suppose that the proteins in the band regions have a particular affinity for nucleic acid, and Wrinch has suggested that this may be due to a higher concentration of basic groups, particularly arginine, which is known to be present in remarkably high amounts in clupein. The difference between the bands and inter-bands appears, however, rather larger and more sharply defined than would be expected if it were due to a merely quantitative difference, but this appearance may turn out to be illusory when

actual measurements of nucleic acid content become available.

The extensibility of the bands seems to differ sharply from that of the interbands, the former being much the more rigid. There are two factors to be taken into consideration here. Firstly, the nucleic acid fiber itself appears to be inelastic, and the rigidity of the bands may be due simply to their nucleic acid content. Secondly, while it is easy to see how nucleic acid may combine with fully extended polypeptides, it is not so clear how it can fit on to a globular protein; it is possible then that the proteins of the bands, when combined with nucleic acid, are in the extended form, and thus have themselves lost much of their extensibility; but whether this should be regarded as a result or as a contributory cause of their affinity for nucleic acid is as yet quite unknown.

The differential staining behavior of heterochromatic regions presumably depends on a chemical composition or physical state different to that of the euchromatin, but in general very little is known about this. In salivary glands of *Drosophila*, the inert regions show a structure of longitudinal striations and transverse bands which is somewhat similar to that of the euchromatic regions, except that the bands are more feebly staining and the whole structure less clear-cut. Although the inert region of X chromosome, for instance, is fairly short in the salivary gland chromosomes and has only a small number of bands, it occupies a large proportion of the whole chromosome at metaphase of mitosis. This may perhaps be partly due to a lesser degree of spiralization in mitosis, although, since the region at that time is definitely shorter than it is in salivary chromosomes, some spiralization must occur. It is probable that the large relative volume of the inert region in mitosis is at least partly due to an abnormally large concentration of nucleic acid on to it at this stage. Muller claims that the greater bulk of the mitotic inert region is produced under the influence of only two loci in

the region, and it is conceivable that the region contains loci specially concerned with the synthesis of nucleic acid.

The physical and chemical basis of this structure is unknown, but it is remarkable to find that the conditions underlying it appear to be transmissible; when inert regions are brought, by translocation, into contact with euchromatic parts of the chromosomes, there is a tendency for the latter to be modified in their appearance in salivary glands, so as to assume more nearly the inert structure. This suggests that the inert regions differ from the euchromatic regions only in some general condition which overlies the same basic differentiation into band and interband.

One physical variable which comes to mind in this connection, but about which we unfortunately know much too little, is the water content. It seems plausible to suggest that changes in hydration of the chromosome proteins occur during the mitotic cycle, perhaps correlated with the hydration changes which are suspected to occur in the proteins of the spindle. Reversible hydration and dehydration of living salivary gland chromosomes has recently been observed under the influence of asphyxiation and changes in osmotic pressure. There are no data known to me as to regional differences in hydration at different parts of the chromosome.

The centromeres are probably quite differently constituted from the rest of the chromosome. They seem to be unable to transmit torsional stresses, since the directions of coiling at metaphase are apparently independent of one another in the two arms of a chromosome with a central centromere; and similarly interference in crossing over does not extend across a centromere. It has also been shown that when centromeres divide at metaphase they do not always split along a plane parallel to the length of the chromosome, but may occasionally be divided transversely or at any angle. All these facts tend to suggest that the centromeres, unlike the rest of the chromosome, are not fibrous structures.

## 4. THE NATURE OF THE GENE

In attempting to work out an adequate picture of the relation between the gene and the chromosome, one can start from the fundamental fact that the chromosome is an elongated structure which, whenever we can analyze it, has differences arranged in a linear order along it; these differences can be detected by linkage studies, chromomere structures, etc. The units, between which differences are noted, may be of different sizes according to the different methods of investigation; there are, in roughly descending order, inert or precociously condensing regions, large chromomeres, ultimate chromomeres or salivary gland chromomeres, and the units of cross-over and x-ray breakage. One might symbolically represent the chromosome thus: abcdefghijklmnopqrstuvw, where there are differences on three scales, between the capitals and lower case letters; underlined, crossed and dashed letters; and finally the letters themselves. The smallest units in this scheme, symbolized by the individual letters, are the units of crossing over and x-ray breakage, and probably measure, as we have seen, about 100 m $\mu$  in length.

If we view the chromosome as it were through the other end of the telescope, attempting to build it up from chemical units, we arrive at a somewhat similar scheme of a linear order of units of different orders of magnitude. The ultimate units now are the links in a polypeptide chain, with a length of only 0,334 m $\mu$ . Exactly what the larger units are is more doubtful, but we have a range of possibilities; there are the periodicities along the chains, the repeat units out of which protein crystals are built, the protein molecules such as they exist in solution, and finally virus particles, all of which may be considered as providing suggestions as to the kinds of units which may be involved. These units range in size nearly up to 100 m $\mu$  which we took as an estimate of the smallest units to be considered when we approach the chromosome structure from the other end. It is, then, possible to conceive of the chromosome as a linear array of units, the units them-

selves forming a hierarchy all the way from heterochromatic and euchromatic regions, some tens of thousands of  $m\mu$  long, to polypeptide links only a few tenths of a  $m\mu$  long.

This apparent homogeneity in the type of formal order exemplified by the chromosome on different scales should not tempt one to suppose that other properties may be just as easily conceived of in any of these scales. For instance, it is sometimes suggested that because the nature of one link in a polypeptide chain may chemically affect the properties of a neighboring link, the same type mechanism may explain the phenomenon of position effect. But in the latter case, the influence is between neighboring genes (*i.e.* breakage units) and extends over distances about a thousand times as great as in the former case. No direct analogy between mechanisms of the two phenomena is possible; and in fact no example of a direct chemical influence extending throughout such a distance appears to be known in protein chemistry.

TABLE OF SIZES\*

Vaccinia virus	175	
Rous sarcoma virus	100	
Tobacco mosaic virus	$430 \times 12$ , $3 \times 12$ , 3	
Bushy stunt virus	28	
Haemocyanin molecule	$59 \times 13$ , $2 \times 13$ , 2	
S13 molecule	10 († shape)	
Repeat unit of virus crystal	$15 \times 15 \times 7$	
Haemoglobin molecule	$2, 8 \times 0$ , $6 \times 0$ , 6	
Protein fibre (repeat unit)	0, $334 \times 0$ , $45 \times 1$ , 0	
Nucleic acid (repeat along fibre)	0, 336	
Gene (estimated maximum dimensions)	$100 \times 20 \times 20$	
Sensitive volumes: gene mutations (Timoffeff-Ressovsky)		c. 1
gene mutations somatic, (Haskins and Enzmann)		15
cytological effects (Marshak)		5

\* The sizes are given in  $m\mu$  ( $= 10^6 \text{ \AA} = 10^{-6} \mu$ ). Where only one dimension is given, it is the diameter of a spherical unit. (Partly after Stanley.)

Certain of the properties of the genes give some hint as to the possible kinds of units which may fill the gap between the  $0,334 m\mu$  polypeptide links and the  $100 m\mu$  genes. The most important is the property of identical reproduc-



tion. Between two cell divisions, each gene causes the formation of another gene exactly like it; if the gene mutates into an abnormal form, it is the mutated gene which is reduplicated. The gene, then, must in some way act as a model on which the new gene is formed. This can only occur if chemical forces originating in the radicals in the gene can extend far enough to influence the nature of radicals formed in the equivalent places in the new gene. The thickness which we can postulate for the gene is therefore limited by the distance through which we can imagine such chemical forces extending. Probably the maximum estimate which is chemically reasonable is about 10 m $\mu$ , which is the order of magnitude of the thickness of the repeat units out of which protein crystals are built. This is of the same order of magnitude as the estimate given above for the maximum thickness of the chromosome thread. It is therefore impossible to reject, from consideration of gene reduplication, the idea that the gene is a single unit. On the other hand, a further difficulty arises in this connection, namely, the necessity to find some mechanism which accounts for the fact that only two genes, the old one and the new, are present at the end of each intermitotic period. The reduplication occurs only once. No plausible hypothesis to account for this has been put forward.

Alternatively, we may assume that the gene is compound, consisting of a number of identical sub-units. Such a supposition probably simplifies the task of accounting for gene reproduction. The chemical forces on which the identity of the new and old gene depend would not have to extend so far from the radicals to which they were due, since the thickness of the sub-units would be less than that estimated for the whole chromosome thread. Similarly, the reproduction might continue gradually, and the gene grow until it eventually split into two by reason of some instability which increased with increasing size, such as that which causes a drop to break up when it passes a certain size limit. The difficulty with this hypothesis is

the fact that some genes (though only a few) show more or less equal rates of back and forward mutation.

It appears not unlikely that nucleic acid plays some important role in the process of gene reduplication. For instance, the most rapid synthesis of nucleic acid occurs just before the prophase of mitosis, at the time when the chromosome appears to split or reduplicate. Again, it is remarkable that the virus proteins which share with the genes the property of identical reproduction in living systems, and of mutation, also contain large quantities of nucleic acid. Conceivably there is some connection here with the remarkable fact recently revealed by Schultz and Caspersson, that nucleic acid is in some way connected with the stability of the gene; when parts of the inert region in *Drosophila* are translocated into the euchromatic regions, they frequently cause the neighboring loci to become unstable and undergo somatic mutations which give rise to phenotypic spotting such as that found with other mutable genes; and this instability appears to be correlated with an increase in the nucleic acid content of the corresponding bands in the salivary gland chromosomes.

All the above considerations apply to genes considered as units of crossing over and x-ray breakage. It is quite possible that only a small part of the gene defined in this way is actually active in the control of development. We cannot rule out the possibility that this activity is due to some particular group within the large protein-nucleic acid complex we have been discussing. In fact the small size of the "sensitive volumes" found for particular steps of mutation might suggest that only quite restricted regions are concerned in producing the phenotypic differences between two allelomorphs; but it is well known that there are many uncertainties in the interpretation of the sensitive volume measurements.

On the other hand, it is quite possible that all primary gene products are enzymes and therefore probably proteins, which may be similar in composition to the genes themselves. It would then be in order to suggest a con-

nection between gene activity, in which enzymes were produced and liberated into cytoplasm, and gene reproduction, in which similar bodies were formed but retained in the neighborhood of the chromosome.

It will be apparent from the above discussion that the exact knowledge at our disposal is so meager that very many alternative hypotheses are still possible as to the nature of the chromosome, and the gene in its different senses. However, the enormously important effects of the genes on development, their capacity for identical reproduction, and the fact that they, rather than the cells of an earlier time, seem to be the most ultimate units into which we can analyze living organisms, makes the problem of their constitution one of the most fundamental questions of biochemistry, well worthy of discussion even long before it can be fully answered.

# THE STRUCTURE OF SALIVARY GLAND CHROMOSOMES<sup>1</sup>

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THE present discussion is limited to the euchromatic regions of salivary gland chromosomes and will center about two main subjects. First, because the bands of the giant chromosomes have been shown to be associated with, or to represent directly or indirectly, the genes, a clear understanding of the nature of the elements which go to make up these chromosomes and their bands is a matter of fundamental importance for any study of the physical nature of the hereditary units. The second aspect, closely tied to the first, is the relation between salivary chromosomes and those we see ordinarily in mitosis or meiosis. With these topics in mind, we shall not consider earlier ideas about the origin and structure of salivary chromosomes. In this rapidly developing phase of cytology, with so many workers from diverse fields taking part, it has been found necessary to discard or greatly modify some of the original concepts, and a discussion of these changes, or a consideration of many special details would serve little purpose here.

The first step towards a clearer understanding of the nature of salivary chromosomes was made by Koltzoff and Bridges, independently, when they attempted to account for the great size of these elements in the fruit-fly. They suggested that the giant chromosomes represent uncoiled prophase chromosomes which have undergone a number of longitudinal divisions without an accompanying cleavage of the nucleus or the cytosome. In effect, each salivary chromosome is a bundle of chromonemata; the bands are transverse discs or, as they actually appear, rows of

<sup>1</sup> Presented at the joint symposium on "Chromosome Structure" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 28, 1938.

homologous chromomeres, of which 16 are commonly seen in *D. melanogaster*, and the achromatic spaces between the bands contain the thin strands or threads which connect the chromomeres of the separate chromonemata. We now realize that the size of the chromosomes in the fruit-fly is not satisfactorily accounted for on the assumption of two divisions of the four original chromatids, nor can we regard the 16 strands as univalent chromonemata or single gene strings. Nevertheless, later studies on more favorable material, such as Bauer's observations on *Chironomus*, or the work which Mr. Griffen and I have done on *Simulium* larvae, have demonstrated the essential correctness of the multiple strand concept. Were the direct evidence less convincing than it is, we would be forced to this conclusion by recent researches on developing insect tissues which show the common occurrence of multiple strand or polytene chromosomes.

In the fruit-fly direct evidence for multiple strands is usually restricted to the dash- or dot-like bands in which the separate chromomeres can be easily seen and counted. Only occasionally, and then in stretched areas, can one observe the very fine threads which connect the chromomeres in a linear series. But in species with broader and more loosely synapsed elements, such as *Simulium*, we generally get a clear picture of the structure. Fig. 1 shows the details seen at the surface of a short section of a salivary chromosome in *S. virgatum* after fixing and staining with aceto-carmin. Essentially the same features are seen at all optical levels as one focuses through the chromosome. Each band is made up of one or more rows of chromomeres, which may be small, densely stained and separate, or large, vesiculate and more or less crowded in one transverse plane. In some rows, as at *b*, *d* or *h*, the chromatin surrounding each chromomere may be dense and more or less fused together, while in other rows (as at *c* or *e*) the chromatin is more flocculent or granular and the boundaries of the separate chromomeres more distinct. Within a given row the chromomeres are usually about the

same size, but occasionally one or more units will be larger than the rest.

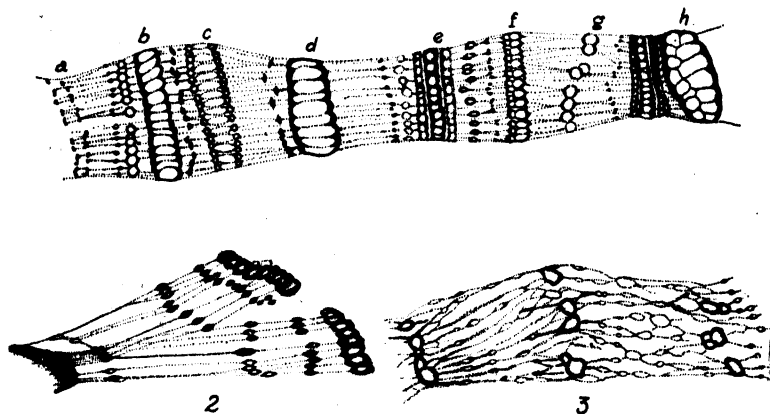
The threads which connect the chromomeres are very fine; they take little or no stain and are best observed in sharply oblique light. Since the four chromatids which go to make up these salivary chromosomes originally are twisted about each other, the fibers run slightly oblique to the longitudinal axis, but generally pass directly from the single chromomeres of one row to those of the next. In *Simulium*, however, not all the bands show the same number of chromomeres, some show about half as many units as their neighbors. In such areas (*d* in Fig. 1) the threads converge in pairs on the (double) chromomeres and diverge (as at *f*) when the chromomeres are again more numerous. The failure of the chromomeres of some bands to divide as often as others is specific, and shows even in the earliest ontogenetic stage.

Fig. 2, drawn at a higher magnification than the other figures, was made from an area where the chromosome was crushed and its elements separated. Here the multiple strand condition and the relation between the thread and its chromomere is very clearly shown. Notice particularly how some of the fibers stick together for a short distance and then separate and pass to single chromomeres. This is not the sort of behavior one would expect were we dealing with stress-lines, but fits in perfectly with the thread and chromomere concept.

The organization shown in Figs. 1 and 2 is typical for all portions of the *Simulium* chromosomes except a short area which we have called the "spread out" region (Fig. 3). Here the structure is more complex. The chromomeres are not arranged in compact bands but are more scattered and the threads connecting chromomeres of various valences form a network much as Metz has described for other forms. Were we to consider the structure of the spread-out region alone, we might well conclude with Dr. Metz that salivary chromosomes are alveolar in nature, but the development of this small area shows it

to be essentially similar to the more characteristic euchromatic portions shown in Figs. 1 and 2.

Turning now to the bands, a point I wish to stress is that many of them are compound in the sense that they are composed of two or more rows of different kinds of chromo-



FIGS. 1-3

Figs. 1 to 3 are from *S. virgatum*. Fig. 1 shows the details seen in a surface view of a fully differentiated salivary chromosome. The various bands, many of which are compound, are made up of discs of homologous ultimate chromomeres which appear as rows in any one plane. Some chromomeres are small densely staining and separate, others large, vesiculate and crowded. Fig. 2 shows a small portion of a salivary chromosome which was crushed in mounting. It is presented to show the relations between the longitudinal fibers and the chromomeres which they connect. Fig. 3 shows the typical structure of the "spread out" region. Here there are no definite bands and the fibers connecting chromomeres of different valences appear to form a spongy network.

meres closely associated linearly (Fig. 8). This feature has important implications and applications for cytogenetics and especially for *Drosophila* workers. In well-fixed and stained salivary chromosomes of *Simulium*, the different rows of chromomeres within a band show with considerable distinctness, but in forms with narrower chromosomes, such as *D. melanogaster*, the individual rows may not show, although many of the bands have been proved compound, both morphologically and genetically (e.g., Muller, Ellenhorn and Prokofieva, 1935). Any one who tries to determine the exact pattern of a short area is apt

to find that the more a given region is stretched, the more bands become distinguishable. Of course the more a chromosome is stretched the greater the chance for the separation of these closely joined chromomere rows, and thus a broad band, in a lax chromosome, may resolve itself into a number of finer bands. Our present chromosome maps for *D. melanogaster* are incomplete and will not be entirely satisfactory until we know the chromomeric constitution of each complex band.

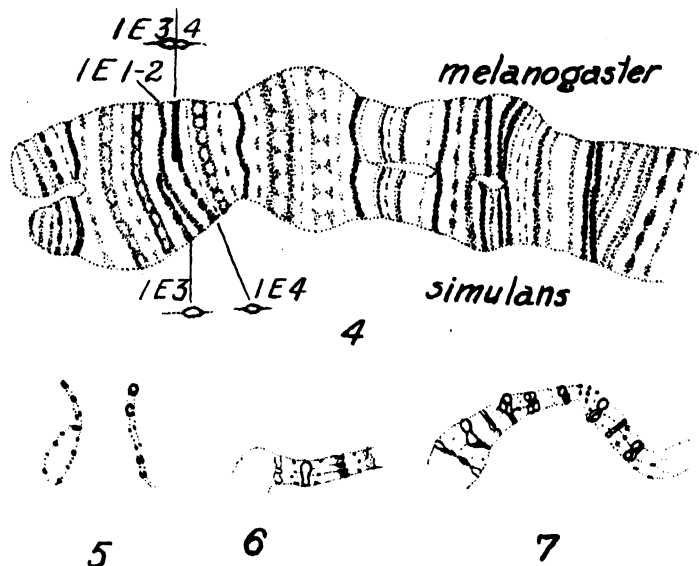
In locating gene loci, in estimating the size and the number of genes, especially in detecting small inversions or deletions, we must recognize the compound nature of many of the bands, and take into account the nature of the individual chromomeres which are involved. Let me illustrate: in hybrid larvae between *D. simulans* and *D. melanogaster*, one of our students, Mr. Horton, has found a tiny inversion in the X-chromosome involving one whole band and a part of another (Fig. 4). In *melanogaster*, the band 1E3-4 appears as a heavy double band. In *simulans* an inversion has divided this "doublet" into a broad and a narrow component. The obvious explanation is that the band 1E3-4 is made up of two rows, at least, of unequal sized chromomeres which were separated by the inversion, but there is no suggestion of this composition of the band in *D. melanogaster*.

A great deal of light has been thrown on the fundamental structure of the giant chromosomes by a study of the way they develop from small nuclei. Workers in the field are pretty generally agreed that the homologous chromosomes which unite to form the salivary chromosomes are split prior to somatic synapsis. Mr. Griffen and I have succeeded in tracing salivary chromosomes back to this four-strand stage. Each of these four chromatids shows the ordinary chromomeric structure of early prophase chromosomes (Fig. 5). The individual chromomeres are tiny, there isn't a great range of size, most of them stain solidly, but a few seem to show hollow or achromatic centers. The fiber which connects these chromo-



meres is extremely fine, it is more the pointed ends of the chromomeres and their linear order which indicates its presence.

The first step in the differentiation process is a growth of the individual chromomeres and not their visible division. As this goes on the sister chromatids and their homologues join in somatic synapsis. In four-strand chro-



FIGS. 4-7

Fig. 4 is taken from a hybrid larva of *D. melanogaster* and *D. simulans* and shows the left-hand end of the X-chromosome. A very small inversion in *simulans* has separated the band 1E3-4 into two components of unequal size showing that this band in *melanogaster* is compound and composed of at least two rows of unequal sized chromomeres. Figs. 5 to 7 are taken from *S. virgatum*. Fig. 5 shows the chromomeric structure of the chromatids from which the salivary chromosomes are formed. Figs. 6 and 7 are taken from 4 strand salivary chromosomes showing the increase in diameter which accompanies the growth of the individual chromomeres.

mosomes there is considerable difference in breadth, as Figs. 6 and 7 will show. As the chromomeres unite by somatic synapsis to form the incipient bands, more and more details become visible. This is due, in some part, to the increase in size which makes the components more easily seen, but a considerable part is due to the spreading

out, probably an uncoiling of the ultimate chromomeres contained within the compound chromomeres of the chromatids which we see earlier. Somatic synapsis seems coincident with the separation of the ultimate chromomeres.

The salivary chromosomes increase rapidly in breadth, and as broader elements are observed we see more chromomeres in the discs or rows and more fibers in the interband area. It is clear that the original chromatids divide and in the end each of the four chromatids we start with contains on the average some 16 strands, making a total of 64 strands per (paired) chromosome. This means that each of the original chromatids has undergone four divisions, a fact that will not account for the great breadth or length of the adult chromosomes. As I have emphasized, it is the hypertrophy of the individual chromomeres which accounts for most of the increase in diameter. Not all the interesting details of development have been worked out yet in *Simulium*, but it is perfectly clear that we start with four chromatids or strands which are chromomeric in nature and end with some 64 strands, each of which is chromomeric in character.

As the salivary chromosomes grow in diameter the shapes of the chromomeres may change in crowded bands. The chromatic hull is the part most affected and in Fig. 8 the sequellae of this crowding process is shown diagrammatically, for units which have a dense rind of chromatin. At the outset, to the left in Fig. 8, the chromatin is more or less evenly distributed about the vesicle. But as the chromomeres become crowded, the chromatin is shifted to the free ends where it unites apparently into a transverse plate. The extent of the shifting of the chromatin depends on the size of the chromomeric vesicles, and thus the appearance of a given band may be quite different in a number of slides. These transverse plates of chromatin have caused Dr. Metz to envision salivary chromosomes as a series of alveoli separated by chromatin discs, a concept which is reasonable enough, if we confine our attention to

fully developed chromosomes, but patently untenable, in view of the developmental history of the salivary chromosomes, if Mr. Griffen and I have correctly recorded it.

Of especial interest, in Fig. 8, is the middle diagram which shows the behavior of the chromatin when homologous chromomeres unite into clusters rather than into transverse rows. Just as in bands, the chromatin tends to lie on the surface of the mass and the boundaries between the individual chromomeres of the ball soon disappear. This rounding up must involve some slipping of the chromomeres so that the enlarged structure is not made up of segments, like an orange, but of many displaced units. This point is of interest from two standpoints. First, it accounts for some of the increase in length of the fully differentiated chromosomes and more important, I think the same process goes on within all chromomeres, as explained below.

What is the nature of the hypertrophy of the chromomeres during the development of salivary chromosomes? To answer this question let us turn to a line of cytological investigation begun before the salivary chromosome era. In 1925 Jacoby showed that in the mouse liver the nuclear volumes do not give a bell-shaped curve, but a series of very sharp peaks, each peak corresponding to an exact doubling of nuclear volume. He interpreted his observations to mean that there had been an "inner division" of the chromosomes without nuclear cleavage as Heidenhain had assumed some years before. Using the same method of study, Hertwig measured the nuclei and computed the volumes of large nurse-cells and salivary gland cells in *D. melanogaster* and concluded that on the basis of nuclear volumes, the chromosomes in salivary glands should show at least 256 strands. This evidence stands in sharp contrast to the 16 strands which we usually see. In *Chironomus*, which presumably is much like *D. melanogaster* in the ranges of nuclear volumes, Bauer finds evidence for two hundred or more strands, which is a nearer approach to the 256 strands expected on the basis of Hertwig's esti-

mates. Buck has studied the increase of nuclear volume in *Sciara* and indicates that the order of increase is a thousand fold or more. I have not made a study of nuclear volumes in *Simulium*, but I have measured and computed the volumes of single early prophase chromomeres and

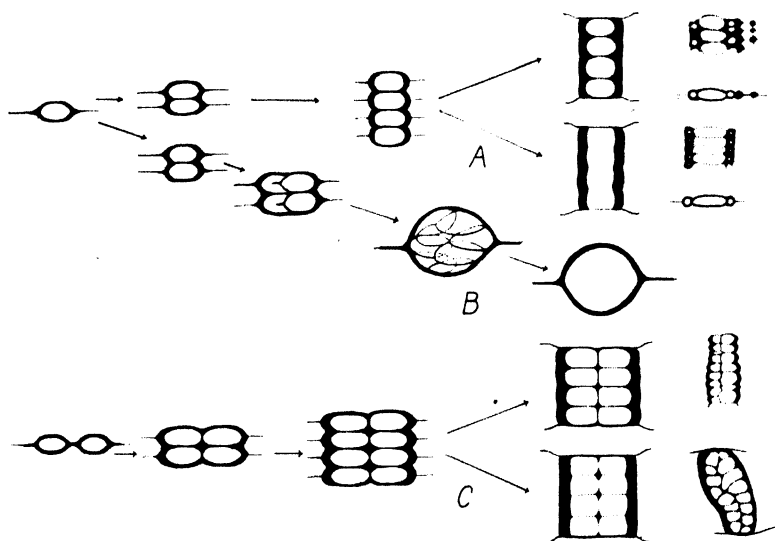


FIG. 8 is a diagram showing the effects of crowding on the distribution of the chromatin in bands when the individual chromomeres are covered by a dense hull of this material. A illustrates the formation of a "double band" both from a single row of chromomeres (on the left) and from three or four rows of non-homologous chromomeres (on the extreme right). B shows how extremely large chromomeres are formed in the "spread out" region. Not only is the chromatin squeezed to the surface of the cluster but there is also some displacement of the separate ultimate chromomeres. C illustrates the way chromatin is displaced when two non-homologous chromomeres are closely associated linearly, and at the right, actual examples of such compound bands taken from *Simulium*.

those seen in a fully developed salivary chromosome and find that the volumes of a single row of chromomeres, in old larvae, is sufficient to encompass 1,984 of the chromomeres seen at the earliest stage. In other words, each of the 64 chromomeres in *Simulium* is sufficiently large to contain 32 of the original chromomeres. It is not to be supposed that such measurements and hence volume calculations are very exact, but they all are more or less in line

and show that the chromomeres we see in fully developed salivary chromosomes of *Simulium*, and other species, must be compound and made up of a considerable number of homologous chromomeres. Whether the number is actually 32, as our measurements suggest for *Simulium*, or more or less, is not so important as the fact that we are not dealing with single ultimate chromomeres in Belling's sense in the salivary glands studied so far, but with aggregates of these.

If we are somewhat hesitant in accepting conclusions based on nuclear volumes, let us consider the work which

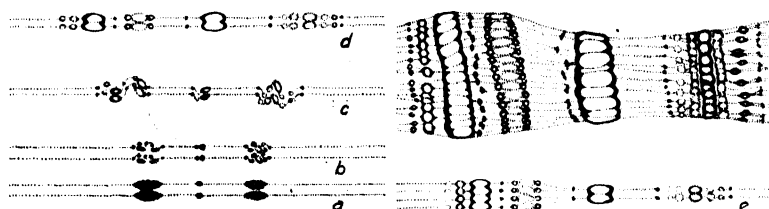


FIG. 9. This diagram illustrates the origin of a number of bands in salivary chromosomes from single compound chromomeres of an ordinary somatic chromosome. Only two chromatids are shown, each with three compound chromomeres (A). Within these compound chromomeres the ultimate chromomeres are arranged probably in a spiral (B). The ultimate chromomeres grow in size and uncoil, thus increasing the length of the salivary chromosome (C and D). Coincident with this somatic synapsis occurs (D). Later the original chromatids divide and ultimately there are an average of about sixteen strands in each of the four chromatids which go to make up salivary chromosomes.

has appeared recently which strikingly verifies the general correctness of our inferences. In the water strider, *Gerris*, we have enormous lobed nuclei in the salivary glands. Geitler (1937) has studied these. In the male, the X-chromosome remains condensed or heteropycnotic during the interphase of mitosis and by counting the number of heteropycnotic elements in a nucleus its chromosome valence, diploid, tetraploid, etc., can be determined. In the giant lobed salivary nuclei, Geitler finds great numbers of these heteropycnotic X's and while exact counts are impossible, he estimates that there must be around a thousand. Most of the larval cells studied so far in insects do not

divide, so that special interest centers about cells with high chromosome counts which can divide, as reported by Berger and by Bauer. The diploid number of chromosomes in the mosquito is 6, but Berger has observed in the mid- and hind-gut of larvae and pupae extremely large nuclei which often undergo division. The highest number of chromosomes which Berger had counted is 192. In the mitoses which follow during metamorphosis, Berger thinks that the chromosome number is reduced again so that in the adult cells nuclei with low valences are the rule. Apparently, Berger has unearthed an ingenious mechanism to hasten the process of metamorphosis and if the development of the mosquito is typical of other insects, we have a possible mechanism for somatic segregation and variegation, in general, without invoking somatic crossing-over.

Bauer has studied the nurse cells of some Diptera in which he finds that as the nucleus grows the chromosomes become polytene. Of especial interest is the fact that these polytene chromosomes unite into a haploid number of bundles which show a banded form. Later these bundles fall apart and many small chromosomes are formed presumably through the coiling of the single chromonemata.

In the light of the foregoing it must be clear that the increase in the size of the chromomeres, during the ontogeny of the giant chromosomes, is a real growth process during which the ultimate chromomeres, in Belling's sense, are reduplicated over and over without a visible subdivision of these units. Of this there can be no doubt, but I hasten to add that in old larvae, there is evidence for the differential swelling (or contraction) of specific chromomere rows to form spindle-shaped, puffed and other swollen areas, and hence in part the final size may be due also to the accumulation of accessory material.

If we grant that the chromomeres we see in later stages are compound, the same evidence forces us to conclude that the single fibers which we see are also compound to the same degree. Thus in *Simulium* we infer that each thread

is made up of some 32 strands. Even these bundles of 32 or more threads are just at the limit of visibility, and it goes without saying that the single univalent chromonemata would be far below the resolving power of our microscopes. In this way the criticism, based largely on molecular consideration, is met that a single gene string could probably not be seen.

There is nothing in the visible structure of the very fine threads between the bands which suggests morphological differentiations such as we might expect were the genes located here. The chromomeres, on the other hand, show a great variety of form, of size and of behavior, and the great constancy of these as well as the linear order, and the evidence from deletions, point to the chromomeres as the places along the chromosome where the genes are to be looked for. The evidence indicates that the chromomeres of salivary chromosomes are not single units and thus assuming that the chromomeres are genes we are not looking at single genes but at aggregates of them, plus, in some cases at least, accessory material.

In *Simulium* the chromomeres range in size from tiny dots lying just at the edge of visibility and thus having a diameter close to  $0.2\ \mu$  up to huge globular masses  $2\ \mu$  or more in long diameter. All the larger units are vesicular, and the chromatin appears to form a rind or hull about the achromatic center. As Caspersson (1935) and others have shown the chromatin is really nucleic acid while the center of each chromomere, as well as the thread, is protein.

How are salivary chromosomes to be compared to ordinary somatic and meiotic elements? In the accompanying Fig. 9 is expressed my concept of the relation between the fully developed salivary chromosomes and the earlier ontogenetic prophase stage. The essential feature is that the chromomeres seen in the chromatids initially are compound and thus give rise to several rows of the ultimate chromomeres which we see in the fully differentiated salivary chromosome. Within the early compound chromo-

meres there is reason to believe that the ultimate chromomeres are coiled, or at least are not in a linear order. As the individual ultimate chromomeres grow in size, the coils loosen up and spread out and coincident with this we have somatic synapsis of homologous chromomeres. The fully developed salivary chromosome, then, is not comparable to a mitotic prophase but rather to an early meiotic prophase, like the pachytene, but instead of four or eight chromatids, we have a much higher number of strands.

Few cytologists will question the compound nature of the chromomeres seen in somatic divisions simply because it has often been shown that the mitotic prophase elements are shorter and show fewer chromomeres than the meiotic prophase. That the internal arrangement of the ultimate chromomeres is a spiral is an inference based on indirect evidence, for we have been unable to detect it by direct observation. A linear orientation of the ultimate chromomeres seems precluded because there would be nothing to prevent somatic synapsis from occurring in any prophase stage. A spiral or irregular arrangement of the ultimate chromomeres within the compound chromomere would prevent the union of homologous units but would allow a general attraction of homologous regions. To my mind, however, the most cogent evidence of such localized minor coils comes from the field of genetics. Chromosome rearrangements, produced either by irradiation or occurring spontaneously, are predominantly small. For example, in hybrid larvae between *D. simulans* and *D. melanogaster*, of the 24 changes seen or deduced, only one is large; the rest are from  $1\frac{1}{2}$  to 10 easily seen bands. Needless to say, the intimate physical contact of the different ultimate chromomeres within the coils of these compound chromomeres would favor minute changes, on either the one or two hit hypothesis.

In presenting the foregoing concept of salivary chromosome structure, I must point out that Dr. Metz and some of his students interpret the features which we all see in fixed and stained preparations in quite a different way.



While he is quite willing to admit that there is probably a very high number of chromonemata in large salivary nuclei, Dr. Metz thinks that the "threads" we actually see are artifacts, stress-lines perhaps, and not bundles of chromonemata, as I would interpret them, nor does he recognize any genetic continuity between the "chromomeres" of fully developed salivary chromosomes and the chromomeres seen in the initial stages of salivary chromosome development. His views are founded on a study of living chromosomes, principally of *Sciara* and *Chironomus*, and on fixed and stained preparations of more or less fully differentiated salivary chromosomes. As yet he has not shown how these chromosomes develop. Adult structure, however, as we all know, is best understood in the light of ontogeny, and the fact that at the beginning, in *Simulium*, the chromatids consist of chromomeres connected by a fine thread and at the end of differentiation you still have visible threads and chromomeres is, to my mind, decisive proof for the concept of salivary chromosome structure which I have put forward.

In studying living chromosomes, however, Metz and his students, principally Buck and Boche, are bringing to light facts which promise to greatly extend our understanding of both salivary chromosomes and other types. I refer to the work which shows that living salivary chromosomes are extremely sensitive to changes in the density of the surrounding medium during which they decrease and increase in volume more than 50 per cent. without apparent injury. Such changes in volume may be accompanied by changes in optical properties, as Shinke (1937) has pointed out. Thus in *Sciara*, which normally shows little evidence of banding in the unaltered living state, the bands show clearly when the salivary gland is placed in salt solutions or in fixatives. Now it is well known that proteins show an extraordinary ability to absorb and give up water reversibly without changing in composition. Recently, for example, Bernol, Fankuchen and Riley (1938) show that crystals of the tomato Bushy Stunt virus swell about 80

per cent. on the addition of water and shrink again the same amount when water is withdrawn. The studies of Caspersson and others have shown that both the threads and the vesicular material of the chromomeres are protein, and the observations of Buck and Boche (1938) may be taken to indicate, in another manner, this same conclusion. In fact, it appears to me as extremely probable that in living salivary chromosomes the proteins must exist in a greatly hydrated state so that the chromomeres are very much larger, than in fixed nuclei, and the fibers swollen into columns of disperse protein micelles. On dehydration, either by the use of salt solution or by fixatives, the extraction of water would cause the protein micelles to lie closer together and when sufficiently contracted, to show as visible fibers. Stretching of the fixed chromosomes might act in a somewhat similar way and cause the more disperse micelle to form more visible aggregates. If I have correctly interpreted this general situation then we have an explanation for a puzzling feature of salivary chromosome structure. We know that the attraction between homologous chromomeres is very strong, so strong that the shape of the entire chromosome may be distorted in order for like units to synapse. And yet, within a chromosome, the very small chromomeres lie quite separate, in fixed preparations. What holds these small chromomeres apart? Obviously, if in the living state the fibers are really hydrated columns of protein, we have a mechanical set-up which would hold the smaller chromomeres apart. Of course, after fixation the attraction no longer exists.

By way of a summary, the salivary gland chromosomes are to be looked upon as bundles of entirely uncoiled chromonemata and thus are comparable to the chromosomes seen early in meiosis. They differ from other polytene chromosomes in the degree of uncoiling which permits the union of homologous ultimate chromomeres, or clusters of these, and other sequellae of somatic synapsis. The visible chromomeres and threads are not to be regarded as

single gene strings, but aggregates of these, the number varying with the age of the chromosome, ontogenetically speaking, and with the species. Nevertheless, the fundamental chromomeric nature of all chromosomes is clearly shown and the large size of the salivary chromosomes and their extended state make them the best material for the application of physical and chemical methods and concepts.

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# CHROMOSOME STRUCTURE AS VIEWED BY A GENETICIST<sup>1</sup>

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I SHALL begin this presentation with the assumption that the A-B-C's of this problem are familiar to my audience and moreover that I am talking to a group who, although they may not be familiar with all the intricacies of our arguments, accept the validity of fundamental concepts developed by geneticists. This stand greatly simplifies my position, since it makes it unnecessary for me to discuss the evidence in support of it by now ancient and well-known postulates such as the evidence that genes are located in chromosomes, that they are arranged in a linear order, that homologous chromosomes may exchange sections through a process called crossing-over. This leaves me free to devote my time to the discussion of concepts which have recently evolved in this rapidly developing branch of biology; to concepts which are more intriguing since they have not yet passed the argumentative stage.

A geneticist is interested in chromosomes because they are carriers of genes and, therefore, his primary interest in the problem under discussion deals with the interrelationship between genes and a chromosome. One of the first questions which arises in connection with this problem is the relationship between the size of chromosomes and the number of genes they contain. Excellent evidence available from genetic and cytological studies of various species of *Drosophila* shows clearly that, as a rule, there is a close parallelism between the number of genes and the length of the chromosomes. In general, small chromosomes carry few genes, medium-sized ones carry more and

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long ones carry still more genes. However, as usual, there are exceptions to every rule. Chromosomes are known which are long in size but at the same time which carry very few genes or no genes at all. Well-analyzed cases of that type are the Y-chromosomes of various species of *Drosophila*. In *D. melanogaster*, for example, the metaphase Y is longer than the X, but X carries almost one hundred times as many known genes as Y. The fact that the relative lengths of these two chromosomes are not the same in all tissues indicates that this is an unusual situation. In salivary glands, for example, the length of Y is only one fiftieth the length of the X, and also the consistency of Y is different from that of the other chromosomes. The whole Y-chromosome, as well as certain regions of some other chromosomes adjacent to the spindle fiber, are heterochromatic. The remaining sections of the chromosomes are euchromatic. In salivary glands euchromatic regions show distinct banding, while heterochromatic sections have loose and not well-defined structure.

Bauer, Demerec and Kaufmann (1938) have found that breaks induced by x-rays are distributed at random within euchromatic sections of salivary gland chromosomes of *D. melanogaster*. They have found also that in heterochromatic regions breaks are more frequent than in euchromatic regions for similar salivary chromosome lengths. However, studies of Kaufmann and Demerec (1937) indicate that breaks in the entirely heterochromatic Y-chromosome occur with approximately the same frequency as in the euchromatic segments of autosomes of equal length if this length is determined on the mitotic metaphase chromosomes rather than on salivary gland chromosomes. From this evidence Kaufmann and Demerec concluded that the metaphase chromosomes approximate more closely than salivary chromosomes the proportional chromonema length of the two regions, and that the frequency of breaks per unit is the same in both. This suggests that the structure of the chromonema is similar in both regions, which is equivalent to saying that this structure is similar

throughout the whole chromosomal complex. This opinion is opposed by Muller and Gershenson (1935), who think that breaks in heterochromatic regions are not distributed at random but are localized to certain places where they occur with higher frequency than in euchromatic regions. They conclude that the heterochromatic regions of X and Y are fundamentally different in structure from the euchromatic regions, and that they consist essentially of non-genic material derived from a very few specific active genes, between which breakage takes place much more readily than between genes in the chromonema of the euchromatic regions. Recent cytogenetic studies on distribution of breaks in the Y-chromosome made by Neuhäus (1938) as well as cytological studies carried on by Kaufmann (unpublished) support the observations of Kaufmann and Demerec (1937), namely, that breaks in the heterochromatic Y-chromosome are distributed at random. These observations are in favor of the assumption that the frequency of breaks per unit of chromonema is similar in all regions of the chromosomes.

Chromosomes, then, may be visualized as composed of two components: (1) of the fiber-like chromonemata which are structurally similar throughout the whole chromosomal complex, and (2) of the material linked to the chromonemata which differentiates various sectors of a chromosome into units called genes by geneticists. This picture has its close counterpart in the picture of the fiber protein molecule as outlined by Astbury and Bell (1938) and discussed here to-day by Waddington (1939). The backbone of the protein molecule may correspond to the fundamental unit of a chromonema, and a group of radicals attached to the backbone may correspond to what we call a gene.

It is of interest to note that the evidence is accumulating which indicates that radicals rich in nucleic acid are associated with the genetically active regions of chromosomes. This is suggested by direct cytological observations of salivary gland chromosomes where chromatin bands are found to be closely connected with genetic loci, and also by

the results obtained with ultra-violet radiation where it was found that the region of the spectrum absorbed by the nucleic acid is the most effective in producing what may be interpreted as genetic changes (Hollaender and Emmons, 1939).

By the use of x-rays it is possible to induce various changes in genes. Many of these changes are lethals, and for some of them it is possible to establish through salivary gland chromosome analysis that they are deficiencies. In some cases this analysis was carried to very small sections, probably to single loci. A study of such deficiencies shows that not all regions of a chromosome are of equal importance to the organism (Demerec, 1935, 1938). It has been found that deficiencies for certain loci are not only lethal to the organism as a whole but that they are lethal even to a small group of cells which would develop as a mosaic patch within the normal tissue (Demerec, 1934; Slizynska, 1938). On the other hand, chromosome regions are known where a deficiency has no detectable influence on the organism (Demerec and Hoover, 1936; Bridges, 1938). Between these two extremes several intermediate stages were detected. For example, deficiencies are known which are lethal to the whole organism but are not lethal to a small group of cells (Demerec, 1934), and some which are not lethal to the organism but manifest themselves as mutant characters (Muller, 1935). That a situation similar to this observed in *Drosophila melanogaster* probably exists in other organisms as well is indicated by results obtained by McClintock (1938), who, in her work with maize, found that the effect on viability of certain deficiencies is not correlated with the length of the deficient segment but rather with the region which is deficient.

What may be responsible for these differences between various loci? It seems probable that new loci originate through the duplication and subsequent differentiation of existing ones. A number of such duplications, which are viable in the homozygote, are on record and they might well be the progenitors of new loci. The most illustrative

case is the Bar duplication cytologically analyzed by Bridges (1936). A good example for the mechanism of origin of duplications is the case of x-ray-induced duplication in tandem described by Kaufmann and Bate (1938). If a small section of a chromosome is duplicated the loci of that section would be present twice and any change or deficiency affecting one gene located in that section would not be expected to show. This would give greater possibility for changes in duplicated genes, since the otherwise detrimental effect of such changes would be neutralized. Also because of the new position of the duplicated section position effect might enter into play and stimulate the differentiation of the genes involved. In the course of time, therefore, duplicated genes would differentiate into new loci which would be essential for the balance of that particular gene system. However, before this final step in the differentiation process is reached intermediate steps would have to be passed. Consequently, in a system where such a process is continuously at work it is to be expected that all stages of differentiation of loci would be represented. In such a case, the cell-lethal loci would be entirely differentiated loci not protected at all by duplications, while the loci with viable deficiencies would be those which are fully protected. The other groups would represent intermediate stages.

One would therefore expect to find an inherent interdependence between genes of a gene system even after they have become differentiated far enough so that their presence is essential for the existence of that system. There is ample evidence available indicating that such interrelationship does exist. The most conclusive evidence of this is the fact that the balance within a gene system is so sensitive that the absence of even one gene out of a total of several thousands may upset it to such an extent that this system is not able to function and the organism does not survive. Moreover, numerous cases of interaction between genes are on record where a change in one gene affects the functioning of another seemingly unrelated



gene. Another striking example of the interdependence of genes within a gene system is available in cases where the stability (mutability) of certain genes is affected by certain other genes. The mutability of the unstable miniature of *D. virilis*, for example, is greatly increased by the presence of any one of the four known genes none of which is located in the same chromosome with miniature (Demerec, 1929, 1930). Rhoades (1938) has found a gene in maize which causes another, otherwise stable gene, to become unstable. Finally Demerec (1937) has described a gene which appears to increase the mutability rate of the whole gene system.

There is ample evidence which indicates that the activity of a certain gene is determined not only by the constitution of the gene system in which it is located but also by the position of the gene within that system. Examples for such position effect are numerous, but Bar is still the clearest demonstration available. As shown by Sturtevant (1925), two Bars, when present in the same chromosome, are more effective than when they are distributed between two chromosomes. A situation similar to Bar was recently described in the case of the dominant Hairy-wing mutant (Demerec and Hoover, 1939). Here also the phenotypic effect of duplication seems to be stronger if the duplicated section is located in one chromosome than when it is divided between two chromosomes. Numerous cases are on record indicating that when chromosomes are broken and reattached in changed positions genes located near breakage points are frequently affected. A recent review of this problem was published by Dobzhansky (1936). Although the possibility of a simultaneous occurrence of a break and a change in a gene adjacent to that break is not excluded, evidence is accumulating (Muller and Prokofyeva, 1935; our unpublished data) which suggests that the phenotypic effect observed in such cases is probably caused by the change in the action of the genes induced by the shift in their position within the gene system.

Interesting evidence of the position effect are various

mottled characters which are readily induced in *Drosophila* by x-rays. Although the mechanism of this mottling may not yet be satisfactorily explained, it is indisputable that, as first noticed by Schultz (1936), all known mottleds are connected with chromosomal rearrangements which have one break in the proximity of the gene exhibiting mottling and the other break in the heterochromatic region. Thus it seems reasonable to assume that certain genes show mottling phenotype when transferred from their normal position into the heterochromatic region. Recent work of Dubinin (1936) indicates that such position effect may extend an appreciable distance along the chromosome.

Considering all this evidence it seems apparent that the activity of a gene is determined by three internal factors: (1) by the chemical constitution of the gene itself, (2) by the genetic constitution of the gene system in which it acts, and (3) by the position of the gene in the gene system. These three internal factors together with the external factors forming the environment determine the phenotype of the organism. A gene, therefore, should be considered as a unit part of a well-organized system, and a chromosome a higher step in that organization. In that sense genes as individual units with fixed properties do not exist, but their existence as component units of a larger system, with properties partially determined by that system, can not be denied.

In concluding, I will summarize salient points of this discussion. As viewed by at least one geneticist, chromosomes are composed of a fiber-like chromonema which is structurally similar throughout the whole chromosomal complex and to which are attached various radicals. A segment of the chromonema with a number of radicals form a molecular unit which is recognizable through its action as a gene. Indications are that genetically active units contain radicals rich in nucleic acid. The whole chromosomal complex forms a sensitively balanced system. The activity of individual units, genes, is determined by their chemical constitution, by the constitution of the whole system and by their position within that system.

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# THE DIMENSIONS AND INTERRELATIONSHIP OF THE RELATIVE GROWTH CONSTANTS

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## THE simple power function

$$y = bx^k \quad (1)$$

has been found to be widely applicable as an empirical representation of the relative growth of two parts ( $x$  and  $y$ ) of an organism, or of a part ( $y$ ) and the whole ( $x$ ). When the equation is applied to the relative growth of the same structures in related types of organisms, characteristic values of the constants  $b$  and  $k$  are obtained for each type. In certain instances it has been found that these constants are inversely related (Hersh, 1931, 1934; Paulian, 1934; Lumer, 1936). Hersh (1931, 1934) has shown further that the relationship is approximately expressed by a decreasing exponential function of the form

$$b = Be^{-rk} \quad (2)$$

where  $B$  and  $r$  are constants.

It was subsequently demonstrated (Lumer, 1936) that if this equation is assumed to be strictly valid, it implies that the relative growth functions in question form a family of curves whose graphs on a double logarithmic grid intersect at a common point with coordinates ( $r$ ,  $\log B$ ). Since the curves obtained by Hersh were found not to conform to this consequence, it was concluded that equation (2) could be regarded as only a crude empirical approximation.

There remains to be answered, however, the question why an inverse relation between  $b$  and  $k$  exists. Clearly, the answer must be sought in the nature of the constants themselves. Since the relative growth function is purely an empirical expression, we are unable to attribute to these constants any significance other than that which they

have by virtue of the mathematical character of the equation. It can be shown, however, that regardless of what their theoretical significance may be, the relationship arises simply from the fact that the constant  $k$  is dimensionally an intrinsic part of the constant  $b$ .

The question of dimensionality in connection with the relative growth function has already been dealt with by Needham (1934), who points out that the two sides of the equation are not dimensionally equivalent. That is, if  $x$  and  $y$  are masses, then  $x^k$  can not in general have the dimensions of mass, and we thus have dimensionally unequivalent terms in the right and left sides of the equation. This means, according to Needham, that the equation "has no true physical meaning, that is to say, no new concept can be deduced from it as it stands, in the sense that the concept of acceleration arises from the relation  $f/M = a$  between mass, force and acceleration" (Needham, 1934, p. 82). This point of view, while not entirely incorrect, is somewhat misleading, since it fails to consider the dimensions of the constants, or at least gives the impression that in order to do so, we must know their physical significance.

Now the importance of the principle of dimensional equivalence lies entirely in the requirement that any equation which correctly describes a relationship between measurable quantities must be expressible in a form valid for measurements in any system of units. An equation expressed in such a form is termed *complete*. It can be shown that a complete equation is also dimensionally homogeneous (*i.e.*, all its terms have the same dimensions) provided that it is the only relationship connecting the variables in question (Bridgman, 1931, pp. 36-42). The restriction of completeness, however, is entirely inconsequential, since, as Bridgman (1931, pp. 14-16) shows, any equation which correctly reproduces the results of measurements made with a particular set of units can be made complete by introducing as a factor with each measured quantity a constant of suitable dimensions. In equation

(1), this amounts to assigning suitable dimensions to  $b$ . By differentiating (1) we obtain

$$\frac{dy/y}{dx/x} = k,$$

from which it is evident that  $k$ , being the ratio of quantities with the same dimensions, is a dimensionless constant, and will therefore not change in value when  $x$  and  $y$  are measured in different units. On the other hand,  $b$  is a dimensional constant which varies with the units of measure employed. Let us suppose that equation (1) has been found to be valid for a particular set of data in which  $x$  and  $y$  are lengths measured in some particular unit, say millimeters, and that  $b$  has been found to have the specific value  $b_0$ . We have

$$y = b_0 x^k.$$

To make this equation complete (*i.e.*, valid when  $x$  and  $y$  are expressed in any other unit of length), we need merely to introduce a factor  $q$ , representing the ratio of the new unit to the original one, in the following manner:

$$yq^{-1} = b_0 q^{-k} x^k.$$

This may be written in the form

$$y = b_0 q^{1-k} x^k$$

from which we have

$$b = b_0 q^{1-k} \quad (3)$$

Thus if the unit of length employed is changed by a factor  $q$ , then  $b$  varies by the factor  $q^{1-k}$ . In other words,  $b$  has the dimensions of  $1-k$  in length.

All this shows that lack of physical meaning on the part of equation (1), in the sense in which Needham uses the expression, is due not to a lack of dimensional equivalence, but rather to the presence of a complex dimensional constant which we are unable to break down into fundamental components. The present discussion does not contribute anything to our knowledge of the significance of the constants  $b$  and  $k$  in terms of fundamental concepts; such

knowledge can be gained only through the development of an adequate theoretical basis for the equation. It merely shows that if the relative growth function is to be a complete equation, then, no matter what their theoretical significance may be, these constants must have the dimensions indicated above.

It is particularly to be noted that  $k$  is dimensionally contained in  $b$ , appearing as a negative exponent. The value of  $b$  depends, therefore, not only on the size of the unit in which  $x$  and  $y$  are measured, but also on the value of  $k$ . Moreover, if all other factors remain constant,  $b$  will decrease in value as  $k$  increases, and we thus have a simple explanation for the existence of an inverse relationship between the values of these constants obtained for related types of organisms. It does not follow that  $b$  and  $k$  must always be inversely related, for the other factors involved need not remain constant. In particular, situations may arise in which  $k$  remains fixed while  $b$  varies, or *vice versa*. Examples of the former are not uncommon in practice (*cf.* Sinnott and Kaiser, 1934), but I am unaware of the existence of any examples of the latter.

There is one additional feature of the relative growth function as it applies to ontogenetic processes which enables us to gain some further insight into the composition of these constants. Both  $x$  and  $y$  are functions of time, and although the time variable does not appear explicitly in equation (1), it is nevertheless present implicitly, since  $x$  and  $y$  are always measured at the same instant of time. It is thus possible, if the time functions are known, to obtain by eliminating the time variable between them a direct relation between  $x$  and  $y$ . This has been done for several of the numerous types of empirical equations which have been applied to the absolute growth of organisms or parts of organisms, and it has been found that in most cases the relation thus obtained is, at least as a first approximation, of the form of (1), with the constants  $b$  and  $k$  expressed in terms of the constants of the absolute growth functions employed (*cf.* Bernstein, 1934; Lumer,

1937; Hamai, 1937; Glaser, 1938). To indicate the general nature of the results, it will be sufficient to consider two examples from Lumer (1937).

A very simple case is that in which each of the parts  $x$  and  $y$  is an exponential function of time. That is,

$$\begin{cases} y = a_1 e^{r_1 t} \\ x = a_2 e^{r_2 t} \end{cases} \quad (4)$$

If each of these is solved for  $t$  and the solutions are equated, we have

$$y = \frac{a_1}{a_2^{r_1/r_2}} x^{r_1/r_2}.$$

This is obviously of the form of (1), with  $k = r_1/r_2$  and  $b = a_1/a_2^k$ . If  $x$  and  $y$  are lengths, then it is readily seen that  $r_1$  and  $r_2$  have the dimensions of (time)<sup>-1</sup>, while  $a_1$  and  $a_2$  have the dimensions of length. It follows that  $k$  is of zero dimensions, while  $b$  has the dimensions of (length)<sup>1-k</sup>.

A second case is that in which  $x$  and  $y$  are simple autocatalytic functions of time, of the form

$$\begin{cases} \log \frac{y}{A_1 - y} = r_1 A_1 (t - t_1) \\ \log \frac{x}{A_2 - x} = r_2 A_2 (t - t_2) \end{cases} \quad (5)$$

in which  $r_1$  and  $r_2$  are the velocity constants,  $A_1$  and  $A_2$  the upper asymptotes, and  $t_1$  and  $t_2$  the times at which the points of inflection occur. The elimination of  $t$  between these equations yields

$$\frac{y}{A_1 - y} = \frac{1}{C} \left( \frac{x}{A_2 - x} \right)^k$$

where  $C = e^{-r_1 A_1 (t_2 - t_1)}$  and  $k = r_1 A_1 / r_2 A_2$ . If  $y$  and  $x$  are small compared to  $A_1$  and  $A_2$ , this equation is approximated by

$$y = (A_1 / C A_2) x^k$$

Here again  $k$  is dimensionless and, since  $C$  can be shown to be dimensionless also,  $b$  has the same dimensions as in the preceding case.



Thus in these cases, as well as in all the others that have been examined,  $b$  can be written in the general form

$$b = PQ_1Q_2^{-k} \quad (6)$$

where  $P$  and  $k$  are dimensionless, and  $Q_1$  and  $Q_2$  have the same dimensions as  $x$  and  $y$ . Now if the quantities  $PQ_1$  and  $Q_2$  are assumed to be constant, equation (6) becomes

$$b = PQ_1e^{-(\log Q_2)k}$$

which is clearly identical in form with (2) if we set  $PQ_1 = B$  and  $\log Q_2 = r$ . On the other hand, if we assume that the relation between  $b$  and  $k$  is expressed by both (2) and (6), then it follows that

$$PQ_1Q_2^{-k} \equiv Be^{-rk}$$

or

$$\log PQ_1 - k \log Q_2 \equiv \log B - rk$$

and hence that  $B = PQ_1$  and  $r = \log Q_2$ . We have, therefore, a necessary and sufficient condition that (2) hold exactly, namely, that  $PQ_1$  and  $Q_2$  have the same values in all the individuals or groups to which the relative growth function is applied. Where  $x$  and  $y$  conform to equations (4), this would mean that  $a_1$  and  $a_2$  are fixed, or, in other words, that each of the two parts has the same  $x$ - or  $y$ -intercept, respectively, in all the cases involved. If the growth of  $x$  and  $y$  is given by equations (5), then we should have  $CA_1$  and  $A_2$  constant.

Success in fitting equation (2) to a set of values of  $b$  and  $k$ , however, can not be regarded as a criterion of the constancy of  $PQ_1$  and  $Q_2$ . Since  $k$  occurs in equation (6) as an exponent, whereas  $P$ ,  $Q_1$  and  $Q_2$  occur as factors, it is evident that fluctuations in  $k$  will affect the value of  $b$  to a much greater degree than will equivalent fluctuations in the remaining parameters. Consequently, a fairly good approximation to (2) may be obtained even when  $PQ_1$  and  $Q_2$  vary considerably. Since under these circumstances  $b$  is a function of four variables, it is impossible without further information to determine the precise

character of the curve which will be obtained by plotting it against  $k$  or against any of the others.

It should be noted that, as can readily be ascertained from (6),  $b$  and  $k$  need not be functionally related; either may remain fixed while the other varies as a function of the remaining parameters. If, however, the two are related, the relation will tend to be an inverse one except in the unusual circumstance that  $P$ ,  $Q_1$  and  $Q_2$  exhibit extremely large fluctuations in comparison with those of  $k$ .

In view of the foregoing considerations, it seems doubtful whether any really useful results can be achieved by investigating empirically the relation between  $b$  and  $k$ . These are both complex entities, whose values depend on those of a number of constituent parameters, the nature of which is at the present time extremely obscure. The present analysis shows that this method of attack will not provide any elucidation of these fundamental constants, since first, the existence of the type of relation which has been found to occur in practice is explicable in terms of dimensional considerations without regard to the theoretical significance of the constants, and second, the ability to fit a particular type of equation to experimentally obtained values of  $b$  and  $k$  does not enable us to draw any conclusions regarding relationships among the constituent parameters.

It should not be inferred, however, that the study of the relation between  $b$  and  $k$  is devoid of biological interest. With increased knowledge of the time relations of ontogenetic processes, such as embryonic segregations, it may be possible eventually to interpret  $b$  and  $k$  in terms of fundamental constants denoting specific characteristics of ontogenesis. Under such circumstances, knowledge of their interrelationship may well prove to be of great importance. In the meantime, while it is at least of interest to determine and to compare such relationships in different groups of organisms, this method of approach will not lead directly to an understanding of the fundamental significance of the relative growth constants.

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# DIFFERENTIAL GROWTH AND EVOLUTION IN A SUBTERRANEAN ISOPOD

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THE purpose of this paper is to present measurements, analysis and discussion of differential growth and its relation to taxonomy and evolution in a subterranean, fresh-water isopod, *Asellus californicus* Miller (1933).

Investigations on the subject of differential growth and its relations to other branches of biology have been greatly stimulated by the publications of Professor Julian S. Huxley, notably his excellent book, "Problems of Relative Growth" (1932), which summarizes the previous work on the subject. Huxley recognizes two types of growth of a part with reference to the body considered as a standard: (1) *heterogonic growth*, in which the growth rate of the part is different from that of the body as a whole, and (2) *isogonic growth*, in which the growth rate of the part and the body are the same. Interstructural heterogony implies changes with age in the relative proportions of parts, a fact of considerable importance in the classification and study of relationships of organisms. There is, however, in practically every instance, a definite relationship between these variables, which may be expressed by Huxley's heterogony formula,

$$y = bx^k$$

where  $y$  is the magnitude of the differentially growing structure,  $x$  is the magnitude of the body or standard of reference, and  $b$  and  $k$  are constants. When  $k$  equals 1, growth is isogonic, and thus isogony is simply a special case of the law of heterogony. Recently, Glaser (1938) has ably reviewed the quantitative relations of growth, time and form.

Preliminary observations on a series of specimens of

*Asellus californicus* (Fig. 1) indicated that certain parts, notably the uropods and second antennae, exhibited disproportionate elongation, with growth, in respect to the rest of the body. It was further noticed that, while the basal segment (protopodite) and inner branch (endopodite) of the uropods both showed this differential growth, the exopodite or outer branch of the uropod appeared to grow more in proportion to the rest of the body. As a result, the exopodite appeared "relatively short and almost rudimentary in the larger specimens" (Miller, 1933) compared to the other parts of the uropod. Other structures also seemed to maintain, with growth, their

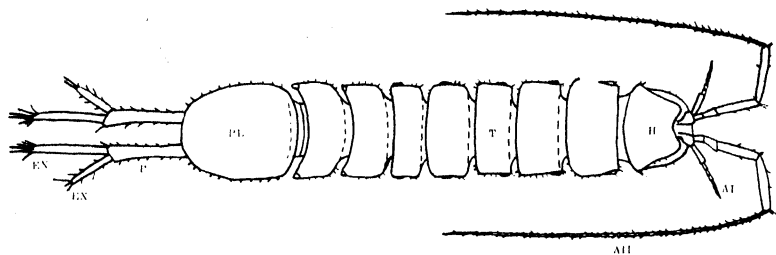


FIG. 1. *Asellus californicus*, dorsal view of male. AI, first antenna; AII, second antenna; H, head; T, third thoracic segment; PL, pleotelson; P, protopodite of uropod; EN, endopodite of uropod; EX, exopodite of uropod.

relative proportions to the body as a whole. Consequently, a series of measurements of various parts of a number of specimens was made and analyzed in order to obtain a better quantitative picture of the growth rates affecting the various parts of the body and their relations to the whole.

The results of these measurements are given in Table I. Measurements were made with a calibrated ocular micrometer and a dissecting binocular.

The first step in the analysis of the data was to determine which of the two measures, body length or body width, was the better standard for comparison of the various structures. Since the coefficient of linear correlation between the two variables was found to be 0.98, it made little difference which we selected. We decided, however,

TABLE I  
*Aesellus californicus*. MEASUREMENTS IN MM.

No.	Sex	Body		First antenna	Second antenna	Head length	Thorax length	Pleo-telson length	Uropod length	Proto-podite length	Endo-podite length	Exopodite length
		Length	Width	Index*								
1	♂	1.5	.3	4.4	.9	.2	1.1	.2	.2	.09	.09	.09
2	♂	1.5	.3	5.0	—	.2	1.1	.3	.2	.09	.1	.09
3	♂	1.9	.4	4.5	1.3	.2	1.3	.4	.3	.1	.2	.1
4	♂	2.3	.4	6.0	—	.3	1.5	.4	.3	.1	.2	.1
5	♂	2.3	.3	6.9	1.1	.3	1.7	.4	.3	.2	.2	.2
6	♂	2.5	.5	5.3	1.6	.4	1.5	.5	.4	.2	.3	.2
7	♂	2.5	.6	4.5	1.7	.3	1.6	.5	.5	.2	.2	.2
8	♂	2.7	.5	5.3	—	.4	1.7	.6	.4	.2	.2	.1
9	♂	2.8	.6	4.3	1.9	.3	1.9	.6	.4	.2	.2	.2
10	♂	3.1	.6	4.8	2.1	.3	2.1	.6	.5	.2	.3	.2
11	♂	3.8	.7	5.3	—	.5	2.5	.9	.8	.4	.4	.3
12	♂	3.8	.7	5.6	2.1	.5	2.6	.8	.7	.3	.4	.3
13	♂	4.0	.8	5.2	2.6	.5	3.0	.9	.8	.4	.4	.3
14	♂	4.3	.8	5.6	—	.4	3.1	1.0	.7	.3	.3	.3
15	♂	4.5	.8	5.5	2.6	.6	3.1	.8	.7	.3	.3	.3
16	♂	4.5	.9	5.2	—	.4	3.4	.8	.7	.7	.3	.3
17	♂	5.2	1.1	4.9	—	.4	3.7	1.1	1.4	.7	.7	.4
18	♂	6.2	1.3	4.8	6.6	1.1	3.7	1.7	2.8	1.5	1.3	.6
19	♂	6.2	1.1	5.6	4.3	.6	4.1	1.3	1.5	1.8	.7	.5
20	♂	7.0	1.3	5.5	—	.6	5.1	1.4	1.9	1.9	.9	.4
21	♂	7.0	1.3	5.5	4.3	.5	5.0	1.5	2.8	1.5	1.3	.5
22	♂	7.3	1.3	5.6	6.0	.6	5.2	1.5	2.3	1.2	1.1	.6
23	♂	7.4	1.3	5.6	8.0	.7	5.3	1.5	2.5	1.3	1.2	.6
24	♂	9.0	1.6	5.7	1.2	.8	6.4	1.8	4.5	2.3	2.2	.7
25	♂	10.9	1.7	6.4	11.1	1.1	7.4	2.3	6.8	3.8	3.0	.8
26	♂	11.1	1.7	6.5	11.9	.9	7.6	2.6	8.3	4.9	3.4	.9

\* Index = length/width in ocular micrometer readings.

that body width was more reliable because the measurements of body length were subject to some error due to differential telescoping of the body segments which are rather loosely articulated. Moreover, the head and pleotelson lengths whose growth rates we planned to study constitute roughly one third of the body length.

We next determined by plotting the values for each organ ( $y$ ) against the corresponding values for body width ( $x$ ) on various types of graph paper that the best linear trends were obtained in each case on a logarithmic graph (Fig. 2). The formula for these straight lines, of course, is  $\log y = \log b + k \log x$ , which is simply another way of writing Huxley's heterogony formula. Thus, our data obey Huxley's law of heterogonic growth.

We notice in Fig. 2, however, that there is a marked increase in slope in the lines for each structure (with the possible exception of the head, thorax and exopodite of the uropod) at approximately 1.0 mm body width representing an abrupt acceleration in the differential growth rates beginning at that period in the life of the animal. These changes in growth rates are like those found by Sandon (1937) in the appendages of the crab *Ocypoda*, but are much more pronounced. We shall hereafter refer to the stable period of growth represented by a change in body width from 0.3 to 1.0 mm as the *first stanza*, and that from 1.0 mm to 1.7 mm as the *second stanza* (following Glaser's terminology, 1938).

The next step was to determine the best-fitting lines to the logarithmically plotted data points, *i.e.*, to calculate the constants  $b$  and  $k$  separately for the two stanzas of growth for each structure. The constants of the best-fitting lines were determined by the method of moments as follows:

$$D = n \sum X^2 - (\sum X)^2$$

$$b = \log^{-1} \frac{(\sum Y \sum X^2 - \sum X \sum XY)}{D}$$

$$k = \frac{n \sum XY - \sum X \sum Y}{D}$$

where  $n$  is the number of cases, and  $X$  and  $Y$  are the logarithms of  $x$  and  $y$ , respectively (formulae adapted from

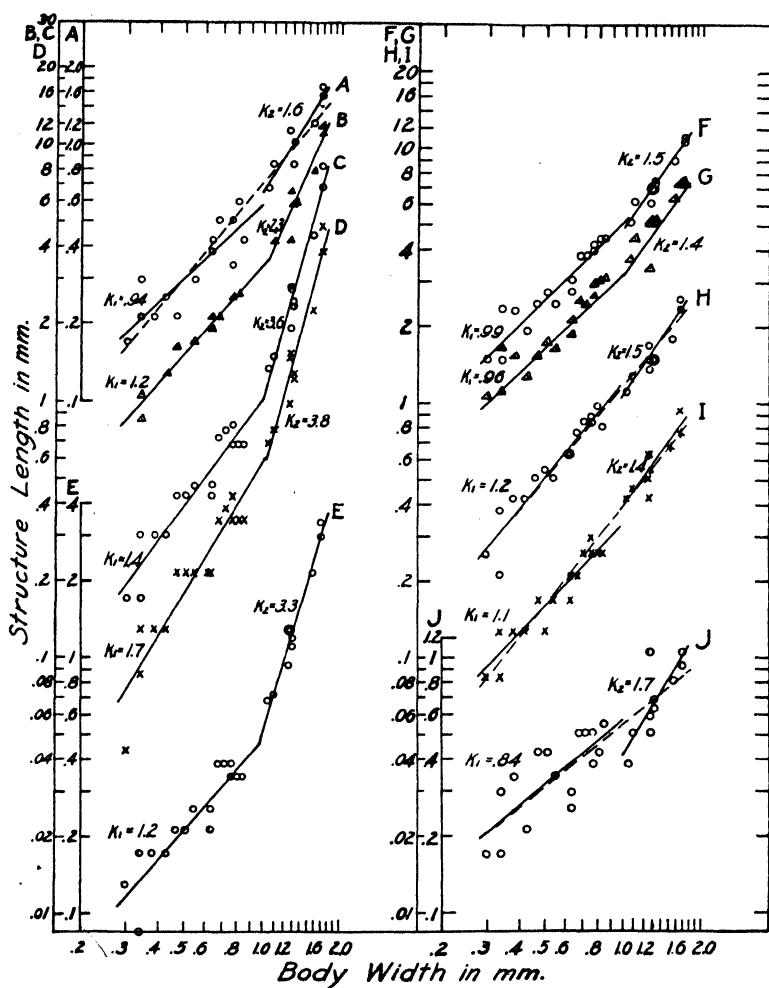


FIG. 2. Logarithmic graphs of structure lengths of (A) first antenna, (B) second antenna, (C) uropod, (D) protopodite of uropod, (E) endopodite of uropod, (F) body, (G) thorax, (H) pleotelson, (I) exopodite of uropod and (J) head plotted as functions of body width in mm. More data near  $x=1$  mm would have closed the gaps between the trend lines of the two stanzas. The dashed lines are the trend lines of the two stanzas combined.

Camp, 1931, p. 106). For those structures whose growth curves did not indicate a large change in growth rates, the constants  $b$  and  $k$  were calculated for the whole range of body widths, as well as separately for each stanza.



The standard errors of  $k$  ( $SE_k$ ) were determined by the following formula,

$$SE_k = \sqrt{n s^2 / D}$$

where  $s$  is given by the following formula, and the other symbols are the same as defined above,

$$s^2 = [n \Sigma Y^2 - (\Sigma Y)^2 - k^2 D] / n(n-2).$$

These two formulae were adapted from Fisher (1936, pp. 137-148) and Snedecor (1937, p. 113).

We next determined which structures, if any, were heterogonic in growth with respect to body width. Since heterogony exists when  $k$  is greater or less than unity, our test for heterogony was simply to determine whether or not  $k$  was significantly different from 1, using  $SE_k$  for the standard error of the difference,  $k - 1$ . If the critical ratio ( $t_{k-1}$ ), i.e., the difference ( $k - 1$ ) divided by the standard error of the difference, was 2.5 or more for eight or more measures, the difference was considered to be significant and growth to be heterogonic. For samples of any size, the difference was considered significant and growth heterogonic if  $P_{k-1}$  was .05 or less, where  $P_{k-1}$  is the probability against the difference being due to chance. (See Table II.—Values of  $P$  interpolated from Fisher's Table IV for values of  $t$  and " $n$ ," where " $n$ " =  $n - 2$ ).

As Huxley has shown, the constant  $b$  has "no particular biological significance since it merely denotes the value of  $y$  when  $x$  equals 1 . . . we may call it the fractional coefficient." By a happy coincidence, the marked changes in growth rates indicated by the change of slope, or the intercepts of the lines in Fig. 2, occur in every case at body width of 1.0 mm, i.e., when  $x$  equals 1. Hence, the values for  $b$  are approximately the same for both stanzas of growth for each organ. In the uropod, for example,  $b$  is 1.00 for the range of body width 0.3 to 1.0 mm and exactly the same for the range 1.0 to 1.7 mm body width (Table II). The fractional coefficient,  $b$ , of course, is necessary in predicting values of one variable given the other.

The constant  $k$ , on the other hand, denotes the constant

TABLE II  
SUMMARY OF GROWTH COEFFICIENTS\*  
 $y = brk$

Structure ( $y$ )	Interval of body width $x$ mm	$b$	$k \pm SE_k$	Critical ratio $t_{k-1}$	Probability against heterogeneity $P_{k-1}$	Critical ratio $t_{k-1}$	Probability against $k_2 - k_1 > 0$ $P$
First antenna .....	0.3-1.0 1.0-1.7 0.3-1.7	0.58 0.66 0.71	$0.94 \pm .15$ $1.6 \pm .25$ $1.2 \pm .077$	0.38 2.48 2.76	.71 .03 .013	1.73	.10
Second antenna .....	0.3-1.0 1.0-1.7	3.41 3.12	$1.2 \pm .085$ $2.3 \pm .36$	1.78 3.76	.12 <.01	3.95	<.01
Body .....	0.3-1.0 1.0-1.7	5.15 4.87	$0.99 \pm .096$ $1.5 \pm .13$	0.077 3.70	>.90 <.01	1.99	.06
Head .....	0.3-1.0 1.0-1.7 0.3-1.7	0.57 0.42 0.55	$0.84 \pm .19$ $1.7 \pm .45$ $0.81 \pm .094$	0.83 1.51 1.97	.42 .18 .06	1.56	.14
Thorax .....	0.3-1.0 1.0-1.7 0.3-1.7	3.41 3.38 3.71	$0.96 \pm .10$ $1.4 \pm .28$ $1.1 \pm .052$	0.45 1.60 1.52	.66 .15 .15	1.66	.11
Pleotelson .....	0.3-1.0 1.0-1.7 0.3-1.7	1.18 1.03 1.14	$1.2 \pm .11$ $1.5 \pm .18$ $1.2 \pm .048$	2.13 2.77 4.13	.05 .03 <.01	0.99	.33
Uropod .....	0.3-1.0 1.0-1.7	1.00 1.00	$1.4 \pm .13$ $3.6 \pm .32$	2.79 8.15	.015 <.01	6.07	<.01
Protopodite of uropod .....	0.3-1.0 1.0-1.7	0.56 0.50	$1.7 \pm .18$ $3.8 \pm .38$	3.83 7.38	<.01 <.01	4.47	<.01
Endopodite of uropod .....	0.3-1.0 1.0-1.7	0.47 0.51	$1.2 \pm .14$ $3.3 \pm .25$	1.09 9.24	.30 <.01	5.91	<.01
Exopodite of uropod .....	0.3-1.0 1.0-1.7 0.3-1.7	0.33 0.39 0.38	$1.1 \pm .097$ $1.4 \pm .27$ $1.3 \pm .054$	0.58 1.53 5.22	.57 .17 <.01	1.23	.23

\*  $b$  and  $k$  were computed from lengths of  $x$  and  $y$  estimated to one place more than those published in Table I and triply checked from original ocular micrometer readings by different methods of analysis.

differential growth ratio of growth coefficient of the structure ( $y$ ) relative to the growth of the body or some standard ( $x$ ) as is evident from the following derivation:

$$\left(\frac{1}{y} \cdot \frac{dy}{dt}\right) = k \left(\frac{1}{x} \cdot \frac{dx}{dt}\right)$$

In words, the relative growth rate of the structure equals  $k$  times the relative growth rate of the standard. The biological importance of  $k$  resides in the fact that, since  $k$  is the exponent in the heterogonic power formula, changes in its value will result in marked visible alterations in the relative proportions of the growing organism, as we shall see later. Graphically represented,  $k$  is the slope or tangent of the angle of inclination of the straight lines on the logarithmic graph: the greater the value of  $k$ , the steeper the slope.

Considering the values of  $k$  in conjunction with their standard errors (Table II), we observe that, during the first stanza of growth, only the uropod and pleotelson are heterogonic in growth. The heterogony of the uropod is due mainly to the marked heterogony of its protopodite ( $k$  equals 1.7), since the length of the uropod is the sum of the protopodite and endopodite. The other structures or parts have  $k$  values not statistically significantly different from 1.0, and hence are isogonic in growth. During the second stanza of growth, however, there is a simultaneous onset of heterogony in each structure previously isogonic, and a still greater heterogony in the uropod as indicated by the astonishingly large and statistically significant<sup>1</sup> increases in the values of  $k$  for this period. In some in-

<sup>1</sup> To test for the statistical significance of these differences between the values of  $k$  in the first and second stanzas of growth ( $k_1$  and  $k_2$ ), we determined the critical ratio ( $t$ ) by dividing the difference ( $k_1 - k_2$ ) by the standard error of the difference ( $SE_{1-2}$ ) which is calculated by the following formulae:

$$SE_{1-2} = s \cdot \left( \frac{n_1}{D_1} + \frac{n_2}{D_2} \right)$$

$$\text{where } s^2 = \frac{(n_1 - 2) s_1^2 + (n_2 - 2) s_2^2}{n_1 + n_2 - 4}$$

When  $t$  is 2.1 or more, for 18 or more measures, the difference is considered

stances, the values of  $k$  are doubled during the second stanza of growth. Indeed, the values of  $k$  for the uropod and its protopodite and endopodite are extremely high for post-embryonic growth of structures. Other structures which show statistically significant changes in  $k$  from the first to the second stanza are the second antenna, and the body (?). The visible consequences of these facts are simply that, during the first stanza of growth, the structures grow more or less in proportion to the body and to each other with the exception of the uropod and pleotelson, which early begin to show relative elongation. During the second stanza of growth, the structures begin to elongate disproportionately with respect to the body. This is especially true in the case of the second antennae and uropods which, in the larger specimens, are extremely elongate relative to the body.

The uropods show in striking fashion the consequences of changing and different values of  $k$  in its constituent parts. Of the parts of the uropod, the protopodite alone is significantly heterogonic in the first stanza, but in the second stanza the endopodite speeds up its relative growth rate and contributes almost equally with the protopodite to the extreme heterogony of the uropod. As a matter of fact, the difference between  $k$  for the protopodite and endopodite in the second stanza is not significant, and hence these two structures constitute, with respect to each other, an isogonic diad. In contrast, the exopodite is isogonic in the first stanza, and only moderately heterogonic in the second stanza. (Although  $P_{k-1}$  for the exopodite in the second stanza indicates 17 chances out of 100 against its  $k$  of 1.4 being different from 1.0, we can still guarantee heterogony in the second stanza, since the  $k$  of 1.3 for the whole range or both stanzas together proves to be significantly heterogonic.) As a consequence of these changing

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significant; or better, when the corresponding  $P$  values (probabilities against significance) are less than .05. " $n$ " in Fisher's table, in this case, is  $(n_1 - 2) + (n_2 - 2)$ . The same formulae were also used in determining significance of differences between any two  $k$  values in Table II, regardless of stanza or structure.

and different values of  $k$ , the proportionate lengths of protopodite, endopodite and exopodite change roughly from 4:6:5, respectively, in the smallest specimens to about 6:4:1 in the largest (Fig. 3).

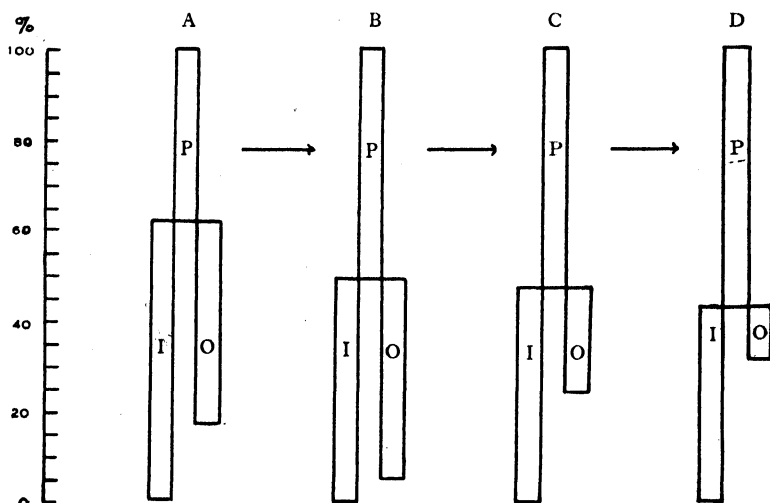


FIG. 3. Diagram showing effects of different growth coefficients ( $k$ ) on the relative proportions of parts of the uropod in *Asellus californicus*.  $P$ , protopodite;  $I$ , inner branch (endopodite);  $O$ , outer branch (exopodite);  $A$ ,  $B$ ,  $C$  and  $D$ , average percentage proportions of the uropods of specimens Nos. 1-3, 13-15, 18-20, 24-26, respectively.

The differences in growth between the first and second antennae are slight, not significant ( $t = .99$ ) in the first stanza, but are fairly marked in the second stanza. Prior to our observations, there must have been differences in the growth of these two appendages or in the time at which growth started since, in the smallest specimens measured, the second antennae were already four times longer than the first antennae, (Table I, Nos. 1 and 3). During the first stanza the ratio between the lengths of the two antennae increases but slightly, if at all. With the advent of the greater heterogony exhibited by the second antennae in the second stanza, however, the ratio between the lengths of the two antennae becomes progressively greater until in the largest specimen measured (Table I, No. 26), the second antenna is seven times the length of

the first. That it should be the second antenna instead of the first which suffers the extreme elongation is not surprising since, in the order Isopoda, the first antennae are always of lesser size and importance (except in the Tanaioidea), and in the land isopods (Oniscoidea) the first antennae are rudimentary vestiges. With respect to the body, the second antennae change from six tenths the body length in the smallest specimen to equal the body length in the largest specimens.

The head is rather variable in length measures, especially in the first stanza, as indicated by the scattering of the data points (Fig. 2) and the large standard errors of  $k$  (Table II). The growth tendency in the first stanza is toward negative heterogony, since  $k$  is less than 1, but this value is definitely not significantly different from 1. Growth changes in the second stanza to positive heterogony, but again we can not statistically guarantee heterogony, or that the change from stanza one to stanza two is significant.

The growth rate in length of the body is conditioned by the growth rates of its constituent parts with the head and pleotelson together contributing about one third, and the thorax the other two thirds. In the first stanza, the tendency toward negative heterogony in the head is balanced by the positive heterogony in the pleotelson, and, since  $k$  for the thorax is 1.0, we should expect  $k$  for the body length to be about 1.0, which indeed it is. In the second stanza, the positive heterogony of the head, abdomen and possibly the thorax make the body as a whole heterogonic in length. The differences of  $k$  between the head, thorax and abdomen are not statistically significant in the second stanza, and hence these structures, although heterogonic with respect to body width, constitute an isogonic triad.

The general pattern of growth in the long axis, in spite of differential heterogony, is orderly, as illustrated by the simple growth gradients in Fig. 4. The protopodite of the uropod is a center of differential growth intensity in both stanzas, extremely high in the second stanza with the endopodite as a close rival. In the second stanza, the second

antenna is also a center of differential growth, and, from these two centers in the opposite terminal appendages of the body, the gradient of "growth potential" slopes proximally down to the middle of the body. We might crudely picture the animal in the second stanza as being stretched out by its ends with the central part of the body relatively inelastic. It is of some interest that the exopodite which is articulated to the protopodite at a considerable angle

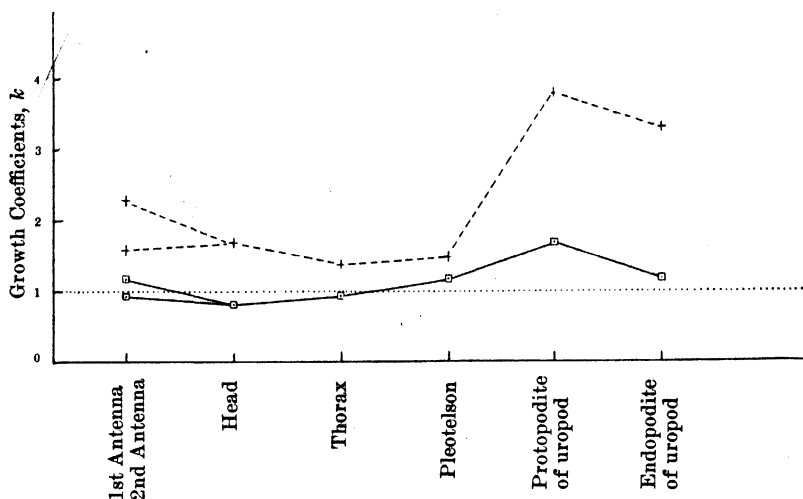


FIG. 4. Growth gradients in the longitudinal axis, *Asellus californicus*. Solid line, first stanza; dash line, second stanza; dotted line, line of isogony.

to the long axis of the body (see Fig. 1) is practically isogonic throughout, whereas the protopodite and endopodite which parallel the long axis of the body are strongly heterogonic.

The most plausible explanation for the existence of stabilized disproportionate growth is that the growth rates of each structure is determined wholly, or in part, by different rate genes. The synchronous onset of heterogony and the existence of orderly patterns of growth, *i.e.*, the simple growth gradients, indicates the probability that there are also genetic factors controlling growth of the organism as a whole, which express themselves at a certain period late in the life of the animal. Through the work of Goldschmidt and other physiological geneticists

we are learning more and more of the existence and behavior of genes determining rates of processes and of the timed action of genes.

The possibility that the heterogony of the second stanza may be a male secondary sexual or sex-limited character is strongly indicated by the fact that all the specimens in the second stanza of growth are males (Table I). This may be due to the small size of the sample or it may indicate a real sexual difference. In the latter case, the onset of heterogony may be coincident with or conditioned by the sexual maturity of the male. Unfortunately, we do not know when sexual maturity occurs in either sex. None of the females was carrying young in the brood pouch, and the small males show apparently as complete development of the second pleopod (copulatory organ in the Asellidae) as the larger ones.

The possibility that the extreme heterogony of the second antennae and uropods may be due, at least in part, to a functional hypertrophy in compensation for loss of eyes can not be excluded, since both of these structures undoubtedly subserve a tactile as well as other sensory functions. That the heterogony of these organs is not entirely due to functional hypertrophy, however, is evident from the fact that the disproportionate elongation is not gradual but increases sharply as we have seen when the animal reaches a certain size. But the functional necessity of sensory contact with the environment is presumably of equal intensity, at least throughout the span of life here studied, and hence there is no apparent functional explanation of the sharp increase characterizing the second stanza of growth. Rather, the synchronous onset of heterogony and the fact that non-adaptive structures are also involved suggests intrinsic factors.

The elongation of the body, especially of the ends of the body and their terminal appendages, is of special evolutionary significance because this feature is one of the striking ways in which subterranean Crustacea differ from the surface species. All the subterranean Asellidae are like our *Asellus californicus* in that they are more elongate



with longer heads, pleotelsons, antennae and uropods than their surface relatives. They also resemble each other in absence of eyes (or rudimentary eyes) and loss of pigmentation resulting in albinism. Elongation, at least of the tactile organs, and the other features mentioned are more or less obviously adaptations to the similar and unique conditions of existence characteristic of subterranean environments the world over (stygian darkness, uniform cold temperatures, etc.). The superficial resemblance of the subterranean asellids to each other resulting from like adaptation to similar environment camouflages their real relationships to different surface species in the environs of their habitats, and obscures the fact that the underground species have arisen independently and at different times in various parts of the world. We have here a clear case of convergence in evolution.

This brings us face to face with the chaotic status of the classification of the subterranean asellids. To review the situation briefly, the subterranean asellid species of the United States and Japan, undoubtedly polyphyletic in origin, are artificially grouped together under the generic name *Caecidotea* Packard, 1871, which is poorly distinguished from the genus *Asellus*, the chief differences being the above-mentioned convergent characters. The subterranean asellids of the rest of the world, possessing the same convergent characters, are properly recognized and classified as belonging to diverse lines of *Asellus*. *Caecidotea* has been declared invalid by various authors (for review see Miller, 1933) on the grounds that its species have arisen independently and at different times from various species of *Asellus*, that its species are more closely related to neighboring surface species than to each other, that its characters are convergent characters not indicative of true relationships, and that even these characters are not good diagnostic or distinctive features, since they intergrade with surface species. If further reasons for invalidating *Caecidotea* were necessary, we might add that, judging from our findings in *Asellus californicus* (which possesses the characters ascribed to *Caecidotea*),

the relative elongation of the body, its parts and appendages, which is one of the main distinguishing features of *Caecidotea*, is not a constant character, but it changes with age in individuals of the same species due to heterogonic growth.

Even in making distinctions between species, our studies have shown that relative length proportions of parts are not reliable characters in this group since heterogony may be at work. But we could give as distinctive taxonomic characters for heterogonic parts "their growth constants. . . . and the absolute size at which heterogony (if not continuous or uniform) begins" (Huxley). We do not anticipate that this refinement will be generally employed, however, because of the difficulty of ascertaining these values as compared to the ease in finding the usual and more obvious qualitative characters. Furthermore, a sample of about twenty-five specimens representative of the growing period is necessary to give us accurate values of the growth constants, but such a sample is comparatively rare in our collecting experience. Many are the species described from a single specimen or a fragment, and a great many more from just a few specimens. We do caution against the common, bald use of relative proportions of parts as important specific characters when heterogony is suspected.

Our final interest in heterogonic elongation is in attempting to determine how it might have originated in *Asellus californicus* and other subterranean forms. If our assumption is correct that the rate of growth is gene-determined, we might further assume that parallel mutations in these rate genes in the various ancestral species resulted in the positive heterogonic elongation of the subterranean species. Assuming the onset of heterogony observed in *A. californicus* to be the expression of the mutant rate genes, the mutation expresses itself relatively late (i.e., at the beginning of the second stanza) in development, as is often the case with recent mutations.

Whatever the cause of heterogony, the result is a recapitulation in that the young subterraneans are more like

the present-day adult surface species and supposed ancestral species in body proportions than are the older specimens. The uropods in *Asellus californicus* illustrate this recapitulation very nicely. In the younger specimens, the basal segment of the uropod is relatively short, its two branches subequal in length (Table I, Nos. 1 and 2), which is the primitive condition of this biramous appendage and like the uropods in the adults of the majority of surface species of *Asellus*. As the animals grow older, the uropods diverge more and more from the ancestral or surface species type, especially with the onset of unequal heterogonic growth in its parts. Judging from the figures of uropods in other subterranean species which show elongated protopodites and endopodites and relatively short exopodites (Van Name, 1936), and from remarks by various authors concerning variation with age in the comparative lengths of these parts, we suspect the same phenomenon of recapitulation due to heterogony could be demonstrated in a series of specimens in other subterranean isopods.

A comparative study of the growth rates in subterranean species with those in related surface species would undoubtedly provide us with further information bearing on the problems of the origin and evolution of subterranean Crustacea.

#### SUMMARY

(1) Measurements were made of body length, width and the lengths of antennae, head, thorax, pleotelson and parts of the uropod of twenty-six specimens of *Asellus californicus* ranging in size from 1.5 by 0.3 mm to 11.1 by 1.7 mm in order to obtain a quantitative picture of differential growth in this subterranean isopod.

(2) The data obtained were analyzed and found to conform with Huxley's law of heterogonic growth, using body width as the standard of comparison.

(3) The differential growth ratio ( $k$ ) for each structure was not uniform over the entire range of observation but increased abruptly at the middle of the range, dividing the period of growth studied into two uniform stanzas.

The constants in the heterogony formula were calculated separately for each structure for the two stanzas, as well as for the entire range when the change from first to second stanza was slight or not statistically significant.

(4) Within the range of body width 0.3 to 1.0 mm, growth in length is practically isogonic for all structures except the pleotelson and the uropod, which is heterogonic, due to the marked heterogony in its basal segment.

(5) In the second stanza (body width of 1.0 to 1.7 mm) there is a simultaneous and significant onset of heterogony in all structures, with the possible exception of the thorax, although in some cases the increase in the value of  $k$  from first to second stanza is not significant. Extreme heterogony is exhibited by the protopodite and endopodite of the uropod with  $k$  values of 3.8 and 3.3, respectively.

(6) Heterogony is not exhibited to the same degree in all structures or parts and the visible consequences of differences in the growth coefficient,  $k$ , are described in terms of changing proportions of parts with growth. The best example is the uropod, in which, as a result of different degrees of heterogony in the protopodite and endopodite and the practically isogonic growth of the exopodite, the proportionate lengths of protopodite, endopodite and exopodite change from about 4:6:5, respectively, in the smallest specimens to about 6:4:1 in the largest.

(7) The growth gradient in the longitudinal axis of the body shows, in the first stanza, a center of differential growth in the protopodite of the uropod from which the gradient slopes down proximally to the head and distally to the endopodite. In the second stanza, the growth gradient features two terminal centers of high differential growth, the second antenna and again the protopodite which is now an extremely high center. From these two distal centers, the growth gradient slopes down sharply to the middle of the body. The whole gradient in the second stanza is significantly elevated above the level line of isogonic growth.

(8) Rate genes affecting the various parts of the body and possibly the body as a whole are suggested as the

cause of disproportionate but orderly growth. That the heterogony of the second stanza may be a male secondary sexual or sex-limited character is a strong possibility. Reasons are given to show that functional hypertrophy is an inadequate explanation for the extreme heterogony of the second antennae and uropods.

(9) The taxonomic and evolutionary significance of heterogonic elongation is discussed. Relative elongation is shown to have no systematic value in separating the subterranean isopods as a genus (*Caecidotea*) from the surface forms not only because it is a convergent character but also because it is not even a constant specific character, since relative elongation varies with age in individuals of a species as a result of heterogony. Other reasons for invalidating this unnatural genus are reviewed. The elongation distinguishing subterranean forms from surface species possibly resulted in consequence of parallel mutations of rate genes in the ancestral surface species. If so, the mutant genes express themselves relatively late in our *Asellus californicus*. The heterogonic elongation of parts in this subterranean isopod results in a recapitulation of ancestral body proportions.

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# ON THE MEASUREMENT AND INHERITANCE OF SEXUAL MATURITY IN TURKEYS (MELEAGRIS GALLOPAVO)

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Using age at first egg as the measure of sexual maturity, Hays (1924) found that early sexual maturity in the domestic fowl differed from late sexual maturity by a dominant sex-linked gene *E* and a dominant autosomal gene *E*<sup>1</sup>. Warren (1934) confirmed these results but did not consider it definitely established that there was only one pair each of autosomal and of sex-linked genes involved.

Sexual maturity in the domestic fowl is, according to Lerner and Taylor (1937), best measured by the age at first egg, while in turkeys date of first egg is more closely correlated with egg production than is age at first egg (Asmundson, 1938). This suggests that in the case of turkeys, date of first egg is a more useful measure of sexual maturity than age at first egg. In order to obtain further evidence on this point, the trapnest records available for pedigreed turkeys hatched in 1931 to 1937 were analyzed. This paper presents the results of that analysis, together with data on birds selected for early and late maturity and on the progeny obtained from reciprocal crosses of birds in the early and late maturing groups.

## MEASURING SEXUAL MATURITY

The stock used for the purpose of determining the best method of measuring sexual maturity consisted mostly of pedigreed Bronze turkeys. A few birds belonging to other varieties and crosses between these were included. The proportion of crossbreeds was small; a considerable part of the Bronze hens kept were inbred. The birds were hatched in the years 1931 to 1937 and, with five exceptions, started to lay the year following the year of hatch. These five birds were hatched in 1937 and started to lay late in December of the same year.

Table 1 shows the number of birds available each year. The date of the first hatch is given in days from the first

TABLE 1  
INFLUENCE OF TIME OF HATCH ON AGE AND DATE OF FIRST EGG

Year	1932	1933	1934	1935	1936	1937	1938
Total number of birds .....	61	67	91	74	73	116	116
Date of first hatch .....	103	103	108	86	93	92	96
AGE AT FIRST EGG IN DAYS							
Average age, hatch 1 .....	318.6	338.6	311.2	332.4	326.8	335.3	289.9
Decrease in average age:							
Hatch 1-2 .....	-11.1	-15.1	-9.4	-18.2	-14.8	-18.2	+5.3
Hatch 2-3 .....	-14.4	-17.5	-14.5	-6.1	-15.4	-4.1	-3.7
Hatch 3-4 .....	-12.6	-5.4	-9.8	-11.1	-9.2	-17.2	-10.6
Hatch 4-5 .....	-9.8	-11.5		-16.6		-10.3	
Hatch 5-6 .....	-2.4						
DATE OF FIRST EGG IN DAYS							
Average date, hatch 1 .....	55.7	75.6	54.2	53.4	54.8	61.3	29.9
Change in average date:							
Hatch 1-2 .....	+2.1	-2.1	+4.6	-3.7	-1.8	-4.2	+19.3
Hatch 2-3 .....	+1.1	-2.5	-0.5	+7.4	-1.4	+8.9	+10.3
Hatch 3-4 .....	+3.1	+8.6	+4.2	+2.9	+4.8	-3.2	+3.4
Hatch 4-5 .....	+3.7	+2.5		-3.6		+3.7	
Hatch 5-6 .....	+9.0						

of the year. Thus birds hatched at 103 days were hatched on April 13. Hatches were spaced two weeks apart; hence the last hatch was six to ten weeks later than the first hatch.

Age at first egg is definitely influenced by the date when the birds were hatched. Except in 1938 the age at first egg of the birds in the second hatch decreased on the average by 9.4 to 18.2 days below the average age of the birds in the first hatch. The birds in the last year (1938) were exceptional in that the birds in the first hatch matured at an earlier average age than those in the second hatch. This was due to the fact that the birds out of the early- and late-maturing strains discussed below were not distributed at random. Most of the birds from early-maturing strains were in the first two hatches, while the later-maturing strains were represented in the second and later hatches. Except for 1938 there was a fairly consistent decrease in total average age from the first to the fourth hatch of 33.7 to 39.5 days or an average of 11.2 to 13.2 days for each 14 days' delay in hatching. These figures are calculated from those shown in Table 1. Age at first

egg does not, however, decrease indefinitely with delay in hatching. This is indicated by the results for 1932, where the decrease in age from the fifth to the sixth hatch was only 2.4 days with a corresponding increase in date of first egg.

Date of first egg (in days from the first of the year in which the record was made) is not consistently influenced by date of hatch early in the season. This is particularly true of the first three hatches or up to about the first week in May. After that there is a slight delay in date of first egg, in most cases, with a decided increase after the middle of June for the one year in which data are available.

Correlation coefficients between the date of hatch and age at first egg and date at first egg were computed. Only the data for the years 1933 to 1937 inclusive will be considered here, since the birds were distributed at random through not more than five hatches in these years. The coefficients of correlation for date of hatch and age at first egg were consistently negative ( $r = -.561$  to  $-.911$ ), while there was no consistent significant correlation between date of hatch and date of first egg ( $r = .028$  to  $.466$ ). The regression of age at first egg on date of hatch varied from  $y = -.8018x$  to  $y = -1.0556x$  in the five years 1933 to 1937. The coefficients of correlation confirm the results presented in Table 1, that age at first egg is consistently influenced by date of hatch but that date of first egg is not.

#### INHERITED DIFFERENCES IN SEXUAL MATURITY

The data presented on the two measures of sexual maturity considered indicate that date of first egg is a better measure of sexual maturity than the actual age at first egg. Table 2 presents an analysis of variance of male families for date of first egg and age at first egg in the years 1933 to 1938, inclusive. Only families comprising three or more full sisters were used. It will be observed that no inherited difference could be demonstrated for age at first egg in the first three years, while in the last three years



TABLE 2  
ANALYSIS OF VARIANCE BASED ON FULL SISTER FAMILIES OF THREE  
OR MORE BIRDS

Year	Age at first egg				Date of first egg. The degrees of freedom are the same as given under age at first egg			
	Between means of sires		Within means of sires		F	Between means of sires		F
	Degrees of freedom	Mean square	Degrees of freedom	Mean square		Mean square	Mean square	
1933	4	468.0	62	239.8	2.03*	120.7	45.7	2.64†
1934	5	614.0	77	345.2	1.78*	1209.0	162.4	7.45†
1935	7	928.2	58	791.6	1.17*	1164.4	169.0	6.78†
1936	6	1672.3	48	258.0	6.48†	1008.1	166.5	6.05†
1937	6	938.3	62	168.8	5.56†	550.0	57.3	9.60†
1938	7	3625.1	78	394.8	9.18†	5304.5	162.5	32.64†

\* These values are below the 5 per cent. point for P.

† The value of the 5 per cent. point for P is 2.52.

‡ These values exceed those for the 1 per cent. points for P.

such inherited differences existed. Apparently variance within families became less as a result of inbreeding, while selection increased the variance between families. While the value of F (see Snedecor, 1934) for 1933 is low, it is evident that there were genetic differences in sexual maturity as measured by date of first egg in all the years. Data for age at first egg, when corrected for date of hatch, gave similar results to date of first egg but appeared to have no advantages over the latter as a measure of sexual maturity in the turkey. Date of first egg has, however, definite advantages over age at first egg, since it is little influenced by date of hatch within the average hatching season and can moreover be used to demonstrate genetic differences in sexual maturity in populations that are hatched at random and are so heterozygous that age at first egg can not be used.

Selections were started for early and late maturity in 1933. Several families of the bronze variety were selected and were inbred in an attempt to obtain reasonably homozygous stock. Brother to sister and son to dam matings were made. One line selected for early maturity and one line selected for late maturity gave reasonably good fertility and hatchability, while most of the others had to

be eliminated because of their failure to reproduce themselves satisfactorily. The basis of selection was date of first egg. Complete families were kept, but the number of individuals available was usually small (Table 3.) The

TABLE 3

MEAN DATE OF FIRST EGG IN DAYS FROM THE FIRST OF THE YEAR IN WHICH THE RECORD WAS MADE, TOGETHER WITH THE STANDARD ERROR OF THE MEAN

Year	Early maturing strain		Late maturing strain		<i>Early</i> male × <i>late</i> females		<i>Late</i> male × <i>early</i> females	
	No. of daughters	Mean date	No. of daughters	Mean date	No. of daughters	Mean date	No. of daughters	Mean date
1934	9	42.44 ± 4.53	7	72.71 ± 1.43				
1935	14	45.86 ± 3.74	7	79.29 ± 2.99				
1936	14	46.00 ± 3.32	11	70.36 ± 1.63				
1937	22	58.86 ± 1.61	7	73.57 ± 1.18	8	58.88 ± 3.18	13	69.23 ± 1.14
1937			6	78.17 ± 1.85				
1938	20	44.25 ± 5.06	10	80.10 ± 1.96	11	61.73 ± 2.44	7	69.71 ± 2.19
1938	13	14.85 ± 3.45	5	85.80 ± 2.16				

data in Table 3 are arranged so that each group in any one line is the progeny of a single male. Thus the birds trapped in 1934, 1935 and 1936 were in each year out of one early male and one late male. The same early male that sired all the early-maturing females kept in 1937 was crossed onto the late-maturing females, while the late-maturing male in that line was mated to the early females. It will be observed that the early male that gave the earliest-maturing progeny was not mated to late-maturing females, and hence no  $F_1$  progeny were available from him.

The differences between the early- and late-maturing lines were all highly significant, although the mean date of first egg varied considerably from year to year. In 1937 and 1938 a few female progeny were available from each of two different males of the late-maturing line. There were slight differences between the progeny of these males in each case, but these differences were just under the statistically significant value in 1938 and barely significant in 1937. In 1938 the progeny of two males in the early-maturing line were available. These two male families undoubtedly differed genetically, one being considerably earlier maturing than the other. This shows

that the early-maturing line was still heterozygous after four generations of selection.

Reciprocal crosses between the early- and late-maturing lines were made in 1937 and 1938. The results differed according to the way the cross was made. When an early-maturing male was mated to late-maturing females, the progeny were significantly earlier maturing than when the reciprocal cross was made by mating a late-maturing male to early-maturing females. In 1937 the progeny of the early male x late females matured at the same time as the birds in the early-maturing line; whereas in 1938 the birds out of this cross were later maturing than the early-maturing line. The birds out of the reciprocal cross were earlier maturing than the birds in the late-maturing line. These results, therefore, clearly indicate that there are both sex-linked and autosomal genes involved.

#### DISCUSSION

The fact that both sex-linked and autosomal genes apparently control sexual maturity in turkeys indicates that the hereditary mechanism is similar to that in the domestic fowl (Hays, 1924; Warren, 1934). Perhaps they have a common hereditary mechanism governing sexual maturity which has not been changed fundamentally during the many thousands of years that these species must have been undergoing evolutionary changes separately. In any case, both domesticated turkeys and chickens comprise a mixture of genotypes which represent different combinations of sex-linked and autosomal genes determining sexual maturity.

There is some evidence that more than one pair of sex-linked and of autosomal genes are involved in determining sexual maturity in turkeys. This is indicated by (1) the differences between the date of first egg of the birds out of the matings of the early-maturing male with late-maturing females in 1938 as compared with that of the birds in the early-maturing line, (2) the differences between the progenies of the early-maturing males in 1938,

(3) the apparent differences in the maturity of the daughters of the late-maturing males in 1937 and 1938. More evidence is needed before definite conclusions can be drawn.

The data presented in this paper and elsewhere show that date of first egg is the better measure of sexual maturity in turkeys, while in the case of chickens, age at first egg is the better measure of sexual maturity (Lerner and Taylor, 1937). Chickens usually start to lay at five to seven months of age. Waters (1937) found that chickens reach maximum body weight at about ten months of age or approximately three to five months after they lay their first egg. Unpublished data from the California Agricultural Experiment Station show that turkey hens reach their maximum first year weight at about forty-four weeks or approximately eleven months. This maximum first year weight is reached by both species in the spring, after which there is a decrease in weight during the summer months followed by an increase in weight during the next autumn. In the case of turkeys the age at first egg will usually vary from about eight months to over eleven months with an average of approximately ten months. The turkey therefore starts to lay considerably later than the chicken and at a time just before it has reached the initial or first year peak in weight. The turkey is thus usually physically mature, in the sense that it is nearly full grown, when it starts to lay, whereas the chicken is relatively immature and continues to grow for some time after it starts to lay.

These differences in the average age of the turkey and chicken may be stated in a different way, namely, that the domesticated chicken tends to start laying at a certain age, while turkeys tend to start laying at a certain season of the year. From this it may be inferred that environmental factors play a much more important role in determining the beginning of egg production in turkeys than in chickens. The most important difference between the two species, however, appears to be a difference in the thresh-

old of response to environmental stimuli. In the chicken this threshold appears to be so low that the female starts to lay at an early age. Physical maturity appears to be an important, if not the determining, factor. The turkey, on the other hand, appears to have a high threshold of response. It is sufficiently mature physically to lay long before it usually does. That this is so is indicated by the results of Scott and Payne (1937), and others, who were able, by the use of artificial light, to induce turkeys to lay about two months earlier than birds kept under ordinary conditions. Their results agree with those reported by Rowan (1925) for juncos, and Bissonnette (1930) for starlings and indicate that light, if it is not the only factor, is at least the most important one in the environment which influences sexual maturity (see also Rowan, 1938). Bissonnette and Csech (1938) have also shown that the long periods of daylight to which winter-hatched pheasants are subjected in midsummer will induce them to start laying at an unusually early age. This finding and the observation that turkeys raised in southern California sometimes start to lay in the autumn when less than seven months of age, while this rarely occurs in the northern part of the state, is further proof that at least some species of birds can lay much earlier than they ordinarily do and that they are usually prevented from laying at an early age by an unfavorable environment.

Since light is the most important environmental stimulus to sexual maturity, it may be supposed that the genotype determines the threshold of response to that stimulus. The genotype also presumably determines the age at which the bird becomes sufficiently physically mature to respond to light. If the assumption that the main difference between early and late-maturing strains of turkeys and between turkeys and chickens is a difference in their threshold of response to light, it is tempting to infer that light would not appreciably lower the age at sexual maturity of early-maturing strains of chickens, while in the case of most strains of turkeys artificial light should induce earlier sex-

ual maturity by providing a stimulus well above the threshold required to start the chain of events that lead up to egg production. Nevertheless, in view of the fact that Curtis (1914) has recorded the case of a Barred Plymouth Rock which started to lay when three months old, it would be extremely hazardous to assume that even early-maturing strains of chickens can not be induced to start laying earlier than they usually do by providing the proper stimulus. There is actually no acceptable experimental evidence to show that domesticated chickens will not mature sexually earlier (and start laying earlier) if subjected to artificial light and hence no experimental basis for the assertion that they differ in this regard from wild birds (Rowan, 1938) and from turkeys. The weight of chickens and turkeys at the time they start to lay when compared with their maximum first-year weight does, however, indicate that physical maturity is ordinarily a more important factor in determining the onset of egg production in chickens than in the case of turkeys.

#### SUMMARY

Statistical analysis of the trapnest records of turkeys extending over seven years shows that date of first egg is a better measure of sexual maturity in turkeys than age at first egg. Date of first egg is little influenced by variation in the time of hatch within the normal hatching season of almost two months, whereas age at first egg is influenced, the later-hatched birds starting to lay when younger than the earlier-hatched birds. Moreover, genetic differences in sexual maturity can more readily be demonstrated with date of first egg than with age at first egg.

Selection for early and late sexual maturity was effective in establishing lines that differed significantly in date of first egg. When these lines were crossed the results differed according to which way the cross was made. The progeny of *late* male x *early* female laid earlier than the birds in the late-maturing line but later than the progeny

of the reciprocal cross. In at least one case, the progeny of the latter (*early* male x *late* female) laid just as early as the birds in the early-maturing line. It is concluded from this that sexual maturity in turkeys is determined by both sex-linked and autosomal genes.

The body weight of turkeys at the time they lay their first egg is usually near the maximum first-year weight, whereas chickens start to lay three to five months before they reach maximum first-year body weight. It is suggested that physical maturity is a less important factor in determining the onset of egg production in turkeys than in chickens and that turkeys have a higher threshold of response to environmental stimuli than do chickens.

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# SHORTER ARTICLES AND DISCUSSION

## PRECIPITIN ABSORPTION WITH *DROSOPHILA* ANTIGENS

### INTRODUCTION

THE object of this report is to show the value of precipitin absorption in defining antigenic relations among *Drosophila* species. Many investigators have performed absorption experiments wherein use was made of agglutinating particles such as blood corpuscles or bacteria. Only a limited amount of work has been carried out in which precipitins have been absorbed. Hektoen and Delves (1932), Duncan (1932), Stone and Garrod (1931) and Lamb and Lamb (1935) have found various technics of precipitin absorption useful in determining antigenic relations of various species. In connection with *Drosophila*, Russian investigators have reported that by the absorption of complement fixing antibodies, they have been able to detect immunologically the presence of the Y-chromosome in the XY and XXY individuals of *D. melanogaster* (Levit, Ginsburg, Kalinin and Feinberg, 1936).

Duncan's work (1932) is of particular interest to us here, because he applied the optimal proportion technic, suggested by Dean and Webb (1926), to the precipitin absorption technic. In this way he was able to determine the exact quantities of antigen and antibody necessary for maximum absorption, without the interference of either serum or antigen excess. The present work has been preceded by a series of tests in which the optimal antigen-antibody ratios were determined. In so far as it was possible, the data obtained in the prior work were used in estimating the relative quantities of antigen and antibody necessary for complete absorption.

### MATERIALS AND METHODS

Antisera were prepared in the manner indicated in earlier reports; and antigens were prepared by extracting with saline the ether-insoluble fractions of the flies (Cumley, in press). All antigens were adjusted for equivalent nitrogen contents, as determined by the micro-Kjeldahl test. Antisera and antigens had been passed through Seitz filters, were preserved with Merthiolate Solution, and were kept at ice-box temperatures. Aseptic



precautions were taken in handling reagents, and no growth appeared in any of them during the course of these experiments.

In absorbing an antiserum with an antigen, 2 cc of the desired antiserum dilution were placed with the same quantity of properly diluted antigen. The mixture was placed in the ice-box for 24 hours, after which it was removed and centrifuged at high speed for 7 minutes. The supernatant liquid was pipetted off and tested by the appropriate reagents. All absorptions and subsequent tests were carried out in Wassermann tubes. Although in these experiments time was kept, as is usual for optimal ratio work, the author found that the determination of the optimal ratio in the supernatant fluids, after absorption, was virtually impossible. Duncan reported similar difficulty.

The antisera to *D. caribbea* and *D. virilis* were absorbed with the antigens of *D. caribbea*, *D. virilis*, *D. mulleri* and *D. melanogaster*. Absorption tests could not be performed on additional antisera because of insufficient quantities of reagents. In fact, some of the necessary cross tests could not be made with the above reagents, because of lack of materials. Using quantities previously determined by the optimal ratio work, several proportions of antigen and antiserum were made up, the object having been to carry out absorption at or near the optimal ratio of flocculation. Also, mixtures were made in which the presence of an antigen or antibody excess was known or suspected. Table 1 is a presentation of the antigen-antibody mixtures that were used in the

TABLE 1

Antigen dilutions	<i>D. caribbea</i> antiserum dilutions			<i>D. virilis</i> antiserum dilutions
	1:1	1:2	1:4	1:1
<i>D. caribbea</i> 1:20 .....			×	
1:40 .....			×	×
1:60 .....				×
1:80 .....			×	
<i>D. melanogaster</i> 1:20 .....		×	×	
1:30 .....		×	×	
1:40 .....				
<i>D. mulleri</i> 1:15 .....	×			
1:20 .....	×			×
1:30 .....				×
1:40 .....				×
<i>D. virilis</i> 1:15 .....	×			×
1:20 .....	×			×

absorptions. An "×" denotes the ratio of antigen to antibody in any given mixture. For example, 2 cc of *D. caribbea* antiserum, diluted 1:4, was mixed with 2 cc of *D. caribbea* antigen, diluted 1:20.

Flocculation tests were run on *D. caribbea* antiserum and *D. virilis* antiserum, against their homologous antigens, to determine the end-points of their reactions. The antisera were then divided into separate portions, and each portion was absorbed by a different antigen. As indicated in Table 1, each species antigen was mixed with a given antiserum in ratios which were thought to include and usually extend to each side of the optimal ratio of precipitation. After absorption, each mixture was centrifuged and the supernatant liquid collected. This fluid was then tested against the homologous antigen, in order to determine relatively how much material had been removed by precipitation during the absorption process. The supernatant liquid was also tested against the absorbing antigen, in order to determine whether all the antibody in common with the antigen had been absorbed at that particular antigen-antibody ratio. Insufficiency of reagents prohibited the testing of the supernatant with the antiserum.

#### RESULTS

Table 2 presents the results of these tests. In the table (+) represents slight precipitate; (+++++) represents heavy opaque precipitate; (-) represents no precipitate.

*D. virilis* antiserum. Referring to Table 2: before absorption, *D. virilis* antiserum precipitated with its homologous antigen to an antigen dilution of 1:640. After having been absorbed with *D. caribbea* antigen, the supernatant fluid reacted with the *D. virilis* antigen to an antigen dilution of 1:160. Consequently, *virilis* and *caribbea* hold fractions in common which produce precipitation at relatively high dilutions. By a removal of these fractions, the precipitating material held by *virilis*, to the exclusion of *caribbea*, is considerably less. In like manner, the *virilis* antiserum was absorbed with *D. mulleri* antigen. Upon testing the supernatant liquid with the *virilis* antigen, precipitation occurred only in antigen dilutions 1:20 and 1:30 (?). When *D. virilis* antiserum was absorbed with its homologous antigen, subsequent tests of the supernatant fluid with the same antigen were negative. Therefore, *D. caribbea* and *D. virilis* are seen to



share a relatively small proportion of antigenic constituents, whereas *D. mulleri* and *D. virilis* share a large proportion of antigenic constituents. There are some fractions which *D. virilis* possesses which are not possessed by any one of the other species tested.

*D. caribbea* antiserum: This antiserum originally precipitated with its homologous antigen in dilution 1:1280. After having been absorbed with *D. virilis* antigen, the supernatant solution precipitated with the *D. caribbea* antigen in maximum antigen dilution of 1:320. Likewise, after having been absorbed with *D. mulleri* antigen, the supernatant fluid precipitated with the *D. caribbea* antigen in an antigen dilution of 1:320. Therefore, *caribbea* shares with *virilis* and *mulleri* approximately the same quantities of antigenic materials. These fractions are relatively small when compared with those shared by *caribbea* and *melanogaster*; for after the *caribbea* antiserum was absorbed with *melanogaster* antigen, the supernatant solution reacted to *caribbea* antigen in antigen dilutions no greater than 1:40. As in the case of *D. virilis*, *D. caribbea* possesses biochemical fractions not held in common with any one of the other species tested. The *D. caribbea* antigen did not completely absorb the *caribbea* antibodies in the antigen-antibody ratios indicated in Table 1. For this reason, there was a slight reaction in antigen dilution 1:20 when the supernatant liquid, produced by absorption with the homologous antigen and antiserum, was subsequently tested with that same antigen. This reaction is insignificant.

#### DISCUSSION

An important observation should be made regarding the foregoing remarks: *D. virilis* and *D. mulleri* antigens give the same reactions to *D. caribbea* antiserum, in so far as this particular test is concerned. This does not imply that the same quality of materials is shared by *D. virilis* and *D. mulleri* with *D. caribbea*. Rather it implies that the quantity of materials shared by *virilis* and *caribbea* measured approximately the same as that shared by *mulleri* and *caribbea*. The same conditions would obtain regarding any other pair of antigens with reference to a given antibody. In this connection it may be well to mention the recent serological work of Irwin (1938) with dove and pigeon species. This investigator was able to prove that each species possessed biochemical

fractions not shared with any other species; and that a species possessed fractions which distinguished it from another species, but which in turn it could share with a third species. Presumably, the same situation exists among the *Drosophila* species.

Sturtevant (1921) placed *D. virilis* and *D. mulleri* in the same subgroup, whereas *D. caribbea* and *D. melanogaster* were placed together in another subgroup. He segregated these species into their respective categories on the basis of various morphological criteria, usually accepted as specific. In the present study *D. virilis* is shown to be closely related to *D. mulleri* and distantly related to *D. caribbea*. Likewise, *D. caribbea* is shown to be closely related to *D. melanogaster* and distantly related to *D. virilis* and *D. mulleri*. Obviously, the taxonomic and serologic methods have yielded much the same results. These data are sufficient to point out the particular advantages of this serological technic in elucidating the evolution of the *Drosophila* species.

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EFFECT OF TEMPERATURE UPON THE DEVELOP-  
MENTAL RATE OF THE EMBRYO OF THE DIA-  
MOND BACK TERRAPIN (*MALACLEMYS*  
*CENTRATA* LAT.)<sup>1</sup>

For several summers experiments have been conducted in an effort to discover whether or not the terrapin (*Malaclemys centrata* Lat.) egg follows the general rule of "cold-blooded" eggs in that the rate of development increases with increase of temperatures over a considerable range.

In the earlier studies a number of difficulties were encountered. Among these was the problem of securing freshly laid eggs in sufficient quantity. Although hundreds of eggs were available in the breeding pens, the terrapins were more or less sensitive to observers unless the oviposition had already been started, in which case the terrapin usually completed the act. When a terrapin was observed in the act of egg-laying, she was allowed to complete the process and was removed from the nest as soon as she began to "fill in." In numerous cases, however, the eggs were so thick in the pen that other nests were disturbed and eggs of an earlier laying became mixed with newly laid eggs. This problem was ultimately solved in the following manner. Parts of the laying pens were fenced off and opened 24 hours before the time for the collection of the eggs. The egg bed was closed off after the 24-hour laying period and thoroughly dug. This procedure was repeated at intervals during the laying season. By this means eggs were secured within 24 hours after laying. These were supplemented by eggs taken from uncovered nests. By this method a rather definite beginning point has been established. In some of the experiments a 48-hour period was used, since it gave many more eggs than two 24-hour periods, due apparently to the fact that terrapins lay better in a territory which they have been permitted to explore for a day or two.

Although taken from a single nest, the eggs show a considerable variation as to the degree of development. In a single oviduct of *Chrysemys cinerea* (Cunningham, 1922) stages from early cleav-

<sup>1</sup> The experiments were conducted at the U. S. Fisheries Laboratory at Beaufort, N. C., and the author expresses appreciation to the bureau for the use of its facilities and to Dr. H. F. Prytherch, director, and Mr. Charles Hastel, foreman, for their cooperation.

age to the late blastopore stage were found, and uterine eggs were taken in which the early flexure was beginning. These differences, however, seem to disappear during development and the eggs of a given clutch, as a general rule, appear to hatch at about the same time.

As has been pointed out in other papers, the problem of humidity, especially in artificially heated nests, was a serious one and even yet in sand nests has not been completely solved. At higher constant temperatures the eggs persist in drying out, even at relatively high humidity. This may account for the failure of eggs to develop at a constant high temperature.

Perhaps the most difficult problem was that of determining the end point. In earlier experiments the end point was taken as the time when the terrapin appeared at the surface. This proved to be a very unsatisfactory criterion, since the newly hatched terrapin remained under ground for quite variable periods of time after emergence from the egg. In our latest experiments the eggs were removed from the nest a few days before the calculated time for hatching and were reset on a bed of sand and covered with wet toweling which could be lifted and the eggs examined without disturbing them. The time of pipping the egg and of complete emergence from the shell could be fairly accurately determined. In our latest experiments the eggs were examined twice daily.

Experiments of last summer, reported elsewhere (Cunningham, Woodward and Pridgen, 1939), indicate that the whole of incubation may be carried out in battery jars without sand. Such procedure would lend itself to more careful temperature studies.

Last summer four distinct types of experiments, as related to temperature, were set up: (1) outdoor beds in which the normal environmental conditions existed; (2) indoor beds exposed to constant high temperature; (3) indoor beds with constant medium high temperature; (4) indoor beds at room temperature. The temperature of 1 and 4 were of course variable, that of 1 being regularly higher than 4. Since the results within each experiment, as well as between experiments, are quite concordant they are reported here.

In experiment 1 there were four beds involved. The recording thermometers showed but little difference in the *nest* temperatures. There were 190 eggs involved in this experiment. The time re-

quired between setting and hatching for the 4 groups is shown here.

Pen A. 61 days. All eggs not hatched at this time  
were used for other purposes.

Pen B. 64-68 days.

Pen C. 62-68 days.

Pen D. 60-66 days.

It is quite evident that in nature, under as nearly identical temperature conditions as possible, there is a considerable range of variation as to the time required for the newly laid egg to hatch.

In the second experiment a relatively high temperature of approximately 98° F. was maintained. The temperature never exceeded 105° F. nor fell below 95° F. The upper temperature is well below the maximum tolerance, since the beds in nature have been known to reach a temperature of 115° F. at the egg level without the death of any considerable number of the embryos. The lower level is considerably above the low level of the outdoor beds.

As in all our previous experiments on high temperatures the eggs failed to develop. Although having plenty of moisture, but not an excess, the eggs shriveled as if they were desiccated. From these experiments, which can not be considered conclusive, it would appear that terrapin eggs can not survive continued high temperatures, although they are lower than the maximum to which such eggs are exposed during normal incubation. If the embryos died without desiccation of the egg, one might attribute death to high rate of humidity, since terrapin eggs are rather sensitive to excess moisture.

In the third experiment the eggs were subjected to a fairly constant temperature of 85° F. There were 24 eggs in this lot, of which 23 hatched. The time required was from 61 to 68 days.

The fourth experiment consisted of two sets of eggs run at fluctuating room temperature ranging from 65° F. to about 92° F. The eggs were placed in battery jars without sand and in one experiment provided with distilled water, in the other with tap water (artesian well). The details of this experiment are described elsewhere (Cunningham, Woodward and Pridgen, 1939). There were 24 eggs receiving distilled water, all of which hatched. The range of hatching time was from 61 to 68 days. There were also 24 eggs receiving tap water, two of which were probably



infertile, the others all hatching. The hatching time was from 61 to 68 days.

In earlier experiments, which were not as well controlled, eggs which were never allowed to reach a temperature of 80° F. hatched in approximately the same time as those under normal conditions. Earlier experiments also indicated that development once started could be completely inhibited at ice box temperatures (approximately 55° F.) and embryos could be held in this condition for a period of at least two months. When returned to normal incubation temperatures such eggs develop in an apparently normal manner.

From the data presented it is evident that the rate of development of the diamond back terrapin does not fluctuate with environmental temperature, but that the rate is more or less constant through a wide range of temperatures. This is contrary to the generally accepted idea that in cold-blooded animals the rate of development is relative to the environmental temperature.

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## MATING TYPES AND THEIR INTERACTIONS IN THE CILIATE INFUSORIA<sup>1</sup>

### INTRODUCTION

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THIS program deals with phenomena in Protozoa that are related to, or analogous to, the diversity of sexes. But a wider view than that of sex diversity is here necessary, as you will see. We are to deal with more general phenomena, of which sex diversity is but one example. We are to deal with the differentiation of organisms into diverse classes, two or more than two, such that members of the same class do not unite for reproduction, while members of different classes *do* so unite. We are calling these diverse classes *mating types*; from this point of view the two sexes in higher organisms are mating types.

The program deals with these phenomena in the ciliate infusoria; and particularly but not exclusively in that familiar acquaintance of all of us, and of many that are not zoologists—*Paramecium*, so generally employed as a type for Protozoa.

Before we begin we wish to try to show by demonstration on the screen, or by photography, or both, the remarkable phenomena in which the difference of type manifests itself, in order that you may realize that we are not talking of doubtful or obscure things, but of striking reactions, things

<sup>1</sup> Presented at the joint symposium of the American Society of Zoologists and the Genetics Society of America in conjunction with the American Association for the Advancement of Science at Richmond, Virginia, December 30, 1938.

that "leap to the eye." Individuals that belong to the same mating type—for example, individuals of a single clone, all derived from the same parent by fission—do not conjugate together. But when individuals of two diverse mating types are mixed, they immediately agglutinate—gather together in large clumps in which the individuals are literally stuck together. From these clots the individuals later emerge as pairs, each pair consisting of one individual of each of the two mating types.

We are going first to venture to try to show these things in the living organisms projected on the screen. The animals that we show are *Paramecium bursaria*, the green *Paramecium*.

[Here followed demonstrations on the screen, as follows: (1) Collection of living individuals all belonging to the same mating type: they do not clump together, but remain swimming about singly. (2) A mixture of individuals belonging to two mating types, three to five minutes after the mixture was made. The individuals have clumped together into large masses. (3) A collection of individuals belonging to a single mating type was projected on the screen: to this was added, in full sight of the audience, individuals of another mating type: the two types clumped together immediately into dense aggregations. (4) A later stage of the aggregations, after five or six hours: the clumps were partly disintegrated into small masses, chains of individuals, pairs and single individuals. (5) The condition about 24 hours after the mixture was made: almost all the individuals united in pairs.]

Photographs of four successive stages in the mating behavior are shown in Fig. 1.

For the ciliate Protozoa, observations that may now be interpreted as indicating a differentiation into diverse mating types were first made in the monumental work of Maupas (1889), published just fifty years ago. We shall be pleased if this program may be considered a jubilee celebration of the great work of Maupas, which has so profoundly influenced all work on the biology of Protozoa.

In four species of ciliates—*Leucophrys patula*, *Onychodromus grandis*, *Stylonychia pustulata* and *Loxophyllum fasciola*—Maupas observed that there is no conjugation

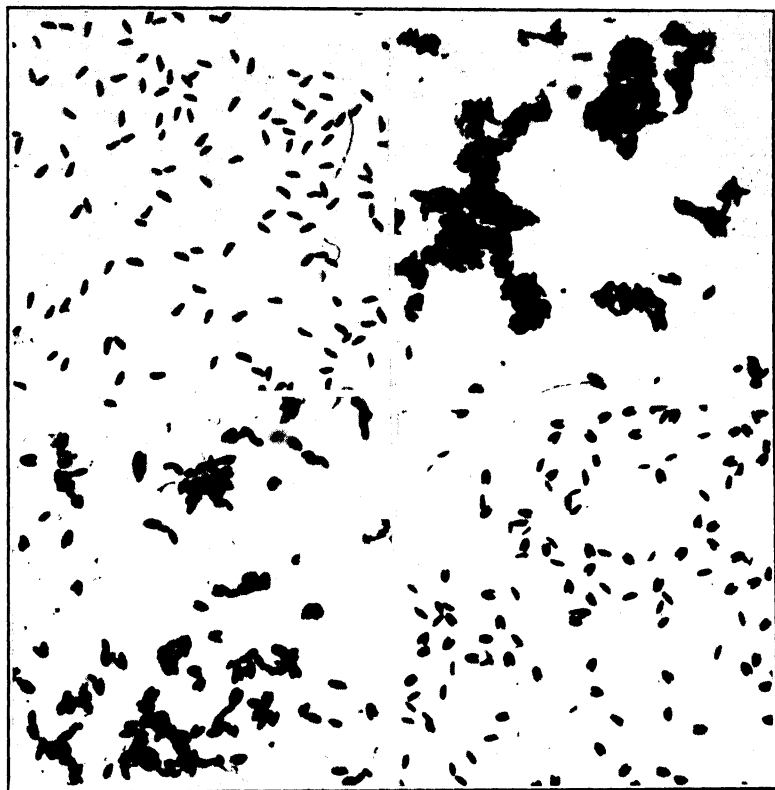


FIG. 1. Photographs of the mating behavior in *Paramecium bursaria*. Upper left, individuals of a single type (C), scattered and swimming singly. Upper right, mixture of individuals of two diverse mating types (P and Q), six minutes after the two were mixed. The individuals clumped into large masses. Lower left, mixture of two types (P and Q) about five hours later. The large masses have disintegrated into small masses, chains and pairs. Lower right, mixture of two types (P and Q) 24 hours after the mixture was made. Most of the individuals in conjugated pairs.

among the descendants by fission of a single individual—among the members of what we now call a *clone*. But if two clones of diverse origin are mixed, conjugation between their members, he observed, commonly occurs.

Maupas believed that this was a matter of closeness of relationship; closely related individuals, he held, do not mate together, while more distantly related ones may do so. As we shall show, this was not an adequate or accurate statement of the situation.

These observations of Maupas appear not to have been taken up and developed for the ciliate infusoria up to the time of the work which we are presenting to-day. I did myself attempt earlier to examine into the matter, but was so unfortunate as to select for intensive study *Paramecium aurelia*. In this species, as it turns out, conditions are exceptional and very different from those described by Maupas. Conjugation frequently occurs between members of the same clone, between individuals showing the closest possible relationship. The situation here could not be understood until knowledge had advanced much farther, in a number of different directions.

This led to a turning aside from the study of these matters. It was only when, in the course of our comprehensive study of the genetics of *Paramecium aurelia*, Sonneborn undertook and carried out a detailed study of the genetic consequences of endomixis, that he discovered the key to the situation; and this opened up the entire subject.

We plan to present to you this morning the main concrete facts and relations that have thus far been discovered in the free-living ciliate infusoria. In that presentation you will observe two general relations that are of interest and that may be stated in general terms at the beginning.

The first is that the conditions found are extremely diverse in different species, and even in different races of the same species.

The second is related to the first; it may be expressed as follows: In any species or race the conditions fall into a system that is clearly marked and unmistakable, a system comparable to that of sex diversity and sex determination in higher organisms. Yet to many features of this system there are exceptions; there occur rare exceptional conditions that do not conform to the system. In a given spe-

cies or race most of the individuals operate in accordance with the system, but occasional individuals do not. Such exceptional conditions are of course not unknown in the sex determination system of higher organisms; in the Protozoa as in higher organisms they are of much interest. They possibly indicate that the systems under study are in a state of evolutionary flux.

One word further as to a wider outlook in these studies. In the unicellular organisms we have the last refuge of "the inheritance of acquired characters." Particular environmental conditions—unusual temperatures, chemicals or the like—produce definite changed characteristics in the organisms, and these "acquired characters" are inherited for many generations of vegetative reproduction; they have been called by Jollos "Dauermodifikationen." These "long-lasting modifications" are commonly, though not always, changed or lost at conjugation or at endomixis. Just what is the nature and seat of these inherited environmental characters? To discover this, we must first work out in full and in detail the normal genetics of these organisms; that is what we are now attempting. Then by crossing modified and unmodified individuals we may hope to discover the secret of the inheritance of these environmental modifications. An understanding of the relation of environmental changes to inherited changes is one of the greatest needs of genetic science. Perhaps the most direct approach to this problem lies in work on the genetics of these unicellular organisms.

For the presentation of these matters, we have prepared a program of two longer papers, dealing with a number of diverse aspects of the subject; then three shorter papers dealing with single aspects of the matter in particular organisms.

# PARAMECIUM AURELIA: MATING TYPES AND GROUPS; LETHAL INTERACTIONS; DETERMINATION AND INHERITANCE<sup>1,2</sup>

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IN *Paramecium aurelia*, the progeny of a single individual do not as a rule conjugate with each other, so long as nuclear reorganizations have not occurred; but when the progeny of certain different individuals are brought together under the proper conditions, they give at once a striking agglutinative sex reaction. Clumping of the individuals is followed by pairing and conjugation. The group of vegetative progeny of one individual, among which conjugation does not ordinarily occur, is called a *caryonide*. Two caryonides that do not conjugate by themselves, but do conjugate when brought together are said to be of diverse *mating types*. Sonneborn (1937) showed that in one stock (S) of *P. aurelia*, all the caryonides were of either one or the other of two mating types, designated I and II.

To ascertain the mating type of a caryonide it is required to mix some of its members with standard cultures of each of the mating types; with one and only one of these it will conjugate: *e.g.*, if it is type I it will conjugate in the mixture with type II, not in the mixture with type I. The mating type of a caryonide, therefore, is the opposite of the type with which it conjugates, the same as the one with which it fails to conjugate.

The composition of the species *P. aurelia* in relation to mating types was ascertained by examining the descendants of representatives of the species isolated from some 30 different ponds and streams in the eastern United States

<sup>1</sup> Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

<sup>2</sup> Nearly all the work of Sonneborn here reported was aided by a grant from the Penrose Fund of the American Philosophical Society.

and California. The progeny of a single wild individual are referred to as a stock. The first problem was to ascertain whether mating types occur in each stock. This was done by obtaining in each stock many caryonide cultures descended from different exconjugant or otherwise reorganized individuals, and mixing samples of each caryonide with a sample of each of the other caryonides. Observations were then made as to which mixtures resulted in conjugation and which did not. The results fell into two diverse systems.

In all the stocks except six, the system of results was like that shown in Table 1 for stock Q. Here, + signifies that the sex reaction and conjugation occurred in mixtures

TABLE 1

*Paramecium aurelia*, stock Q. Results of mixing different caryonides. + = conjugation; - = no conjugation. In each square is given the result of mixing the caryonides on the corresponding row and column.

[illegible]



of the two caryonides represented on the corresponding row and file and — signifies the sex reaction and conjugation did not occur. It appears that the caryonides are of two diverse types; mixture of any caryonide of the one type with any caryonide of the other type results in the sex reaction and conjugation; but mixture of two caryonides of the one type or of two caryonides of the other type does not result in the sex reaction or conjugation. These two kinds of caryonides are thus of two different mating types, and in such a stock every caryonide will react sexually and conjugate with either one or the other of the two mating types; ordinarily no caryonide will react with both types. This general relation is based on the study—in some stocks—of many hundreds of caryonides.

The remaining six stocks show a different system, illustrated by stock B in Table 2. In these stocks the sex reaction and conjugation never occur in mixtures of different caryonides; not merely the 20 caryonides shown in the table, but literally hundreds and thousands of caryonides have been examined with the same result. The explanation of this system of results appears at once when caryonides of such stocks are mixed with the diverse mating types found in certain other stocks. In the case of stock B, for example, all its caryonides give the sex reaction and conjugate when mixed with one of the two mating types found in stock S, type II, but not when mixed with the other one, type I. Hence, *all* caryonides of stock B are of mating type I; they are identical in behavior with type I of stock S: they conjugate with type II, but not with type I. On analysis, it is found that all 6 of the stocks that fail to conjugate when diverse caryonides *within* the stocks are mixed, do conjugate when mixed with one, but not when mixed with the other, mating type in certain stocks.

In sum, of the 36 stocks investigated, 30 consist of two interbreeding mating types and the remaining stocks consist of but a single mating type each. In *P. aurelia*, mating types appear to be of universal occurrence: each caryonide

TABLE 2

*Paramecium aurelia*, stock B. Results of mixing different caryonides with each other and with mating types I and II of stock S. + = conjugation; - = no conjugation. In each square is given the result of mixing the caryonides on the corresponding row and column.

		Stock B CARYONIDES																				Stock S Types	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	I	II
Stock B CARYONIDES	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Stock S Types	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Stock B

in every stock belongs to some definite mating type. There are no non-conjugating stocks or caryonides.

Are there but two mating types among all these stocks? This question was tested directly by mixing the types found in each of 17 of the stocks with the types found in each of the other 16 stocks. The results of these 496 different mixtures are shown in Table 3. It appears that while some stocks contain the same two types, others contain two different types. Altogether, however, only 6 different types exist. In one group of stocks (B, F, J, etc.) the two types, I and II, are found; mating type I from any of these stocks reacts and conjugates with mating type II

TABLE 3

Results of mixing in all possible combinations of two the mating types found in 17 different stocks of *Paramecium aurelia*. The capital letters give the names of the stocks, the Roman numerals designate the mating types. += conjugation; -= no conjugation. In each square is given the results of mixing the stocks and types on the corresponding row and column.

		GROUP 1				GROUP 2								GROUP 3			
		BL	SC	UL	SC	AC	DE	GI	K	L	AV	CV	EV	GV	KV	LV	WV
GROUP 1	TB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	UW	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GROUP 2	AC	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AD	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AE	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AF	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AG	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AI	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AK	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AL	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-
	AV	-	-	-	-	++	++	++	++	++	-	-	-	-	-	-	-
	AW	-	-	-	-	++	++	++	++	++	-	-	-	-	-	-	-
GROUP 3	CV	-	-	-	-	-	-	-	-	-	++	++	++	++	-	-	-
	EV	-	-	-	-	-	-	-	-	-	++	++	++	++	-	-	-
	GV	-	-	-	-	-	-	-	-	-	++	++	++	++	-	-	-
	KV	-	-	-	-	-	-	-	-	-	++	++	++	++	-	-	-
	WV	-	-	-	-	-	-	-	-	-	++	++	++	++	-	-	-

from any stock; but neither of these types will conjugate with the same type from the same or a different stock. Nor will either of these mating types conjugate with any of the other stocks. A second group of stocks (A, C, D, etc.) shows an independent but similar mating system: the two mating types in these stocks are called III and IV, since they are different from (*i.e.*, do not mate with) the types I and II. Mating type III, from any of this second group of stocks, gives the sex reaction and conjugates when mixed with type IV from any of the stocks, but neither of these types will conjugate with the same type from the same or a different stock. Finally, there is a small third group of

stocks (M, Q and Y) with two mating types that do not conjugate with any of the types I, II, III or IV and so require different designations. These two types, V and VI, show again the same system of interbreeding shown by the first two pairs of types: V from any stock mates with VI from any stock, but no two V's or two VI's will interbreed.

The system of mating types shown by these 17 stocks is illustrated in condensed form in Table 4. The species

TABLE 4

The system of mating types in *Paramecium aurelia*. The six mating types fall into three groups of two types each, with conjugation only between the two types of the same group, never between different groups.  
+ = conjugation ; - = no conjugation.

			Group or variety					
			1		2		3	
			I	II	III	IV	V	VI
Group	1	I	-	+	-	-	-	-
		II	+	-	-	-	-	-
or	2	III	-	-	-	+	-	-
		IV	-	-	+	-	-	-
Variety	3	V	-	-	-	-	-	+
		VI	-	-	-	-	+	-

consists of 3 diverse groups of stocks. Each group contains two mating types that interbreed with each other: Group 1 with its mating types I and II; Group 2 with its mating types III and IV; Group 3 with its mating types V and VI. But there is no conjugation between the different groups. However, many stocks exist within each group and most of these contain both the mating types characteristic of the group, a few containing only one of them. This system shows that to test a new stock it is necessary merely to mix its caryonides with each of the six mating types: any one caryonide will react with only one of the six types and all other caryonides of the stock will react with either the same type or with the other type found in the same group. This method of analysis was applied to the remaining 19 stocks. Six of them were thus found to belong to Group 1, 11 of them to Group 2 and 2 of them to Group 3.

The division of the species *P. aurelia* into non-interbreeding groups of mating types agrees with the situation previously found in *P. bursaria* by Jennings (1938).

There is some slight evidence that at least two of the three diverse pairs of mating types from different groups of stocks of *P. aurelia* may be derived from one original pair of types. Although no conjugation occurs between the different groups, mixture of type II from Group 1 with type V from Group 3 does result in a very weak sex reaction: pairs form repeatedly for a few moments at a time, then break apart. Thus, types II and VI are similar in that both react with type V; and types I and V are similar in that both react with type II. This suggests that I and V are diverse modifications of one original type, while II and VI are diverse modifications of another original type. No such relation has been found between any other two types from different groups.

The existence of three non-interbreeding groups of stocks raises the question of whether each group is a distinct species. If the groups differed consistently in any morphological respects, the answer would be clear; but such differences have not been observed. All conform to the description of *P. aurelia*. Even in the absence of such differences, the thoroughgoing sexual isolation of the three groups is held by some geneticists to be sufficient ground for considering them as distinct species. Such a stand, however, affords serious practical difficulties for investigators not in a position to make the experiments necessary for identification. I therefore propose to consider the three diverse groups of stocks as three varieties of *P. aurelia*, varieties 1, 2 and 3. As will appear, the three varieties differ in a number of physiological respects.

The most striking differences among them are in the conditions required for conjugation: the necessary conditions of temperature and time of day differ for the three varieties.

Variety 1, with mating types I and II, gives the sex reaction and conjugates at any hour of the day and at any temperature within the range examined (9°–32° C.).

Variety 2, with mating types III and IV, will give the sex reaction and begin to conjugate only at temperatures below 25°, best below 20°, and only between 6 P.M. and 10 A.M., best between 1 A.M. and 5 A.M. Variety 3, with mating types V and VI, will give the sex reaction and conjugate only below 28°, best below 24° C., and only between 1 A.M. and 1 P.M., best between 4 A.M. and 11 A.M. In variety 3, it has been shown that this diurnal periodicity is a consequence of the daily alternation of light and darkness. After 5 days of continuous darkness, mating reactions occur equally well at any hour of the day or night; after 5 days of continuous illumination, the mating reaction will not occur at any hour of the day or night. That the effective action here is not a stimulation by darkness, but an inhibition of the sex reaction by light is evident from further experiments. When cultures are put into darkness at different hours, they all begin to react at the same time; hence darkness does not stimulate reactivity. When put into darkness at the same time and exposed to light at successive hours next day, the later they are first exposed to light the later they continue to react; hence light suppresses reactivity.

#### LETHAL INTERACTIONS BETWEEN DIVERSE STOCKS

In addition to the varietal differences in conditions required for the sex reaction and conjugation there are also differences with respect to the effects of lethal substances produced by some of the stocks.

When the mating types of different stocks were mixed together by twos in all possible combinations, as earlier described, it was observed that in many of the mixtures there appeared animals abnormal in form, structure and behavior, and ultimately many corpses, so that in these mixtures many of the animals die. The mixtures that showed such phenomena among 26 of the stocks are indicated in Table 5 by L; those that did not, by O. As is evident, only those mixtures which included stocks E, G or H (all three belonging to variety 2) gave these peculiar results. It was further discovered that the same conse-

TABLE 5

*P. aurelia*. Lethal interactions in mixtures of different stocks. L = lethal interaction in mixture of stocks on corresponding row and file. O = no lethal interaction. In the lethal mixtures involving stocks E and G, the animals spin around the longitudinal axis during the days prior to death. In the lethal mixtures involving stock H the animals become sluggish and vacuolated in the hours prior to death. In the mixtures of stocks E and G with stock H, the H lethal effect appears, not the E or G effect.

VARIETY	1										2										3							
	Stocks	B	F	J	N	O	P	R	S	T	Z	A	C	D	E	G	H	I	K	L	U	V	W	X	M	Q	Y	
1	B	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	F	O	O	O	O	O	O	O	O	O		O	O	O	L	L	L	O	O	O	O	O	O	O	O			
	J	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	N	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	O	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	P	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	R	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	S	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	T	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
Z	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O	
2	A	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	C	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O	O
	D	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	E	L	L	L	L	L	L	L	L	L	L	L	L	O	O	L	L			L	L	L	L	L	L	L	L	
	G	L	L	L	L	L	L	L	L	L	L	L	L	O	O	L	L	L	L	L	L	L	L	L	L	L	L	
	H	L	L	L	L	L	L	L	L	L	L	L	O	L	L	L	O	L	O	O	O	O	O	O	L	L	L	
	I	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O
	K	O	O	O	O	O	O	O	O	O	O	O	O	O		L	O	O	O	O	O	O	O	O	O	O	O	O
	L	O	O	O	O	O	O	O	O	O	O	O	O	O		L	L	O	O	O	O	O	O	O	O	O	O	O
	U	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O	O
	V	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O	O
W	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O	O	
X	O	O	O	O	O	O	O	O	O	O	O	O	O	L	L	O	O	O	O	O	O	O	O	O	O	O	O	
3	M	O		O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O	O
	Q	O		O	O	O	O	O	O	O	O	O	O	L	L	L	O		O	O	O	O	O	O	O	O	O	O
	Y	O		O	O	O	O	O	O	O	O	O	O	O	L	L	L	O	O	O	O	O	O	O	O	O	O	O

quences developed if the other stocks were put in fluid in which stocks E, G or H had lived. These effects were shown not to be due to any contaminant in the fluid, for thorough washing of the animals of stocks E, G and H was followed by the production of lethal fluid again. Moreover, the lethal power of such fluid quickly wore off in the absence of animals of the three stocks involved: its activity was lost in 3 days at 10° C., in 1 day at 30° C., in 1 minute at 50° C. Obviously, the animals themselves of stocks E, G and H make the fluid in which they live lethal to other stocks. The lethal substances require different times to

produce their first visible effects: 2 to 4 hours in the case of the one produced by stocks E and G, 12 to 18 hours in the case of the one produced by stock H; each produces characteristic symptoms; to each there are hereditary differences in reaction and in susceptibility; each is active in very great dilutions.

The E and G lethal substances appear to be identical in their effects. They make animals stop feeding, develop many crystals in the cytoplasm, and spin vigorously on their longitudinal axes. Within a few days, all die, even if they are washed free of the lethal fluid and carefully cultured.

The H lethal substance produces very different effects. It makes animals of susceptible races stop feeding, become sluggish and badly vacuolated and die within 36 hours.

The two kinds of lethal substance not only produce diverse effects on the same race; but each lethal substance produces somewhat diverse effects on the different races and varieties of the same species. The E and G lethal substance acts quickly and violently on varieties 1 and 3 of *P. aurelia*, but very slowly and slightly on variety 2. It is easy to discover within 24 hours whether or not a new and unknown stock belongs to variety 2 by this simple test without making the elaborate preparations and tests to discover its mating types and conditions for conjugations. The H lethal substance also acts quickly and strongly on varieties 1 and 3 and also on a few races of variety 2; but most races of variety 2 show varying degrees of lesser susceptibility, and some are completely immune. If an unknown new race shows little or no effect of the H lethal substance—and this is discoverable in 24 hours—it is certain that it belongs to variety 2. Thus, variety 2 is characterized by including races that can produce these lethal substances and usually by a high resistance or complete immunity to them; while varieties 1 and 3 apparently do not produce lethal substances and are highly susceptible to them. Similar differences in reaction and sensitivity to these two lethal substances have been found among other



species of *Paramecium* and, in general, resemble similar interactions known among the Fungi (see review by Porter and Carter, 1938).

These lethal interactions throw light on certain problems of ecology and evolution. They show that certain stocks of the same species and certain combinations of species could not coexist in nature. Varieties 1 and 3 of *P. aurelia* are thus at a great disadvantage in the struggle for existence, as compared with variety 2; and this is perhaps an explanation of the fact that the majority of natural collections of *P. aurelia* consist exclusively of variety 2.

#### DETERMINATION AND INHERITANCE OF MATING TYPE

From the foregoing, it is evident that discovery of mating types in *P. aurelia* has led to a radical change in knowledge of the composition of the species and of the interactions between its component races and varieties. It is also leading to equally radical changes in knowledge of heredity in this species. The genetic analysis has been pursued farthest in variety 1, so that most of the following account refers to this variety only. On certain points, results obtained with variety 3 are given for comparison; but variety 2 is left entirely out of consideration, for it has not yet been sufficiently investigated from a genetic point of view.

The inheritance of mating type follows different rules and shows different features in genetically diverse material. It is therefore necessary to take up separately: (1) stocks that contain two mating types, (2) stocks that contain only one mating type, and (3) hybrids between these two kinds of stocks and their descendants.

(1) *Stocks that contain two mating types.* In stocks containing two mating types, the types are ordinarily strictly inherited without change within the caryonide, *i.e.*, during vegetative reproduction. To this general rule exceptions of great interest occur rarely. These have been investigated by Kimball; an account of his discoveries will be set forth later. I turn now to the inheritance of mating types at nuclear reorganization.

There are at least two kinds of normal nuclear reorganization processes in *P. aurelia*. In one of them, conjugation, two individuals of different mating type unite, fertilize each other and separate. From the fertilization nucleus each exconjugant then develops a new nuclear apparatus which is, therefore, biparental in origin. Another type of nuclear reorganization occurs periodically within single individuals, without the introduction of nuclear material from another individual. In this type of reorganization, according to Woodruff and Erdmann (1914), neither gamete nuclei nor syngamy occur; but Diller (1936) holds that they do. The former call this type of reorganization endomixis, the latter autogamy. To distinguish this type of reorganization from conjugation, without prejudice as to the exact processes involved, it will be referred to as uniparental nuclear reorganization. Perhaps it occurs sometimes in the form of endomixis, sometimes in the form of autogamy. Genetic evidence on this will be presented towards the end of this paper.

At conjugation, the mating types show the same features of inheritance in both variety 1 (types I and II) and variety 3 (types V and VI). Among a set of exconjugant individuals some give rise to progeny all of one mating type, some give rise to progeny all of the other mating type and some give rise to two diverse lines of descent, one of one type and the other of the other type. In those exconjugant individuals that give rise to both mating types, these segregate as a rule at the first exconjugant fission: of the two products of the first fission, one and all its vegetative descendants are of one mating type, while the other and all its vegetative descendants are of the other mating type.

At uniparental nuclear reorganization, Kimball (1937) found the same set of relations in stock S of variety 1: some reorganized individuals yield progeny of one type, some the other type, and some both types. As at conjugation, when the latter occurs, the types segregate at the first fission after the new nuclear apparatus is formed. I have confirmed Kimball's results in other stocks of variety 1 and also in variety 3.

Thus, in these stocks, the mating types arise in essentially the same way after conjugation and after uniparental nuclear reorganization. Further similarities in the rules and ratios of inheritance will appear below. Apparently the introduction of nuclear material from another individual does not influence the phenomena with which we here deal. The determination of mating type appears to be governed by some process common to both conjugation and uniparental nuclear reorganization. The clue to the process involved is given by those reorganized individuals from which two lines of descent differing in mating type arise at the first fission. At the same fission, each of the two resulting individuals receives one of the two new macronuclei normally formed in each reorganized individual. Are the mating types determined by the macronuclei? Critical evidence on this question is afforded by the exceptional relations found in certain stocks.

In stock R of variety 1, the mating types I and II frequently segregate at the *second* fission of the exconjugant, instead of at the first one. If the macronuclei determine the mating types, then there should be more than two new macronuclei formed in those reorganized individuals which yield segregation of mating type at the second fission. In agreement with this, cytological investigation showed that in stock R about 20 per cent. of the reorganized individuals contained more than two new macronuclei: in most cases only three or four, but in a few cases up to ten. In this stock, therefore, it frequently requires two, and very rarely three, fissions before the new macronuclei formed at nuclear reorganization are segregated to different individuals. The frequency with which mating type segregated at the second fission was found to agree with the expected frequency if mating type were dependent upon the macronuclei. This exceptional evidence, together with the normal case, the evidence set forth below in relation to the effect of temperature on the mating type ratios, and other lines of evidence that time does not permit me to review here, make it perfectly clear that the mating types are determined by the macronuclei.

This then is the basis of the term "caryonide." A caryonide is a group of individuals having macronuclei descended by division from a single original macronucleus. Such a group of individuals are ordinarily alike in mating type. Each reorganized individual usually gives rise to two caryonides; in some stocks, occasionally more. The caryonides produced by a single reorganized individual are sometimes alike, sometimes different in mating type. Each caryonide comes to an end at the next nuclear reorganization, when the old macronuclei disintegrate and new ones are formed.

The relative frequency with which caryonides of each mating type appear after nuclear reorganization (both conjugation and uniparental reorganization) differs in different experiments. Table 6 summarizes the results of many

TABLE 6  
*P. aurelia*. Stocks with two mating types.  
Inheritance ratios at conjugation and uniparental nuclear reorganization.

Variety 1							
	Number of caryonides						
Mating Type I . . . .	128	111	391	103	212	15	83
Mating Type II . . . .	63	91	394	133	426	38	279
Ratio II/I . . . . .	0.5	0.8	1.0	1.3	2.0	2.5	3.4

Variety 3							
	Number of caryonides						
Mating Type V . . . .	116	166	103	83	77	25	40
Mating Type VI . . . .	108	202	143	139	157	62	138
Ratio VI/V . . . . .	0.9	1.2	1.4	1.7	2.0	2.5	3.5

such experiments by Kimball (1937) and Sonneborn (1937, 1938a and unpublished). In variety 1, the ratio of type II to type I varies from 0.5 to 3.4 in different experiments, though most of the experiments—as indicated by the larger number of caryonides—give ratios close to 1.0 and 2.0. Likewise, in variety 2, the ratio of type VI to type V varies from 0.9 to 3.5; in this variety, the absence of experiments with a great excess of type V and the absence of any tendency for the ratios to be simple are conspicuous.

What is the basis of these diverse ratios? They do not depend entirely on differences in the genetic constitution of the parents, for the same parent clones gave different ratios in different experiments. Further investigation showed, as appears in Table 7, that the temperature during

TABLE 7  
*P. aurelia*. Stocks with two mating types.  
Effect of temperature on inheritance ratios at conjugation.

Variety 1			
Temperature	Number caryonides		Ratio II/I
	Type I	Type II	
10°-19° C	87	80	0.9
30° C	29	50	1.7

Variety 3			
Temperature	Number caryonides		Ratio VI/V
	Type V	Type VI	
10°-14° C	138	153	1.1
18°-30° C	128	278	2.2

Variety 3				
Temperature during Conjugation Reorganization		Number of caryonides		Ratio VI/V
		Type V	Type VI	
10°	10°	66	64	1.0
20°	10°	50	44	0.9
10°	20°	27	62	2.3
20°	20°	31	62	2.0
20°	30°	29	64	2.2
30°	20°	21	73	3.5
30°	30°	36	96	2.7

conjugation and subsequent nuclear reorganization is one important factor. Both the ratio of type II to type I and type VI to type V is twice as great at higher temperatures as at lower ones.

Further analysis, in variety 3, throws some light on the period of nuclear activity that is sensitive to the different temperatures. The low ratio characteristic of low temperatures appears when the temperature is 10° C. during the period of nuclear reorganization (*i.e.*, between the time that conjugation is completed and the time that each conjugant undergoes its first fission) and is independent of the temperature during conjugation itself. On the other hand, the high ratios obtained at high temperatures appear when the temperature during conjugation is 30° C., regard-

less of what it is thereafter. It appears likely that factors other than temperature also influence the mating type ratios.

The temperature effective period in all these experiments ends with the first fission after nuclear reorganization. By this time the mating type has become fixed; no changes are producible by further changes of temperature. The type previously determined is inherited absolutely through all succeeding fissions until the next nuclear reorganization. As the temperature has been a factor in determining the mating type during nuclear reorganization, we have here a demonstration of the effectiveness of an environmental condition in determining an inherited character. From the evidence presented above, demonstrating that the mating types are determined directly by the macronucleus, it is to be inferred that the environment acts on the macronucleus so as to "set" it for one type, and that when so set, it reproduces thereafter true to this type independently of later environmental conditions. In agreement with this, the temperature effective period is the period in which the macronuclei and the micronuclei that give rise to them are developing. In this striking example of the inheritance of environmental effects, we are dealing with inheritance through a type of nucleus peculiar to the ciliate Protozoa, the macronucleus, which may be characterized as a purely somatic nucleus, persisting and active only during vegetative reproduction and disappearing at conjugation and uniparental nuclear reorganization. These environmental effects therefore are not passed on through sexual reproduction, but only through vegetative reproduction and are of significance only in uniparental genetics, not in biparental genetics.

Results of the kind just set forth appear likely eventually to lead to a better understanding of one of the most perplexing chapters in protozoan genetics. In an extensive series of investigations on *Paramecium*, Jollos (1921) developed the concept of "Dauermodificationen," subsequently extended to other lower and higher organisms.

These long-lasting environmentally induced modifications, persistent sometimes through hundreds of fissions, were observed to appear and disappear and sometimes to reappear at conjugations and uniparental reorganizations. In some respects, the parallel to the phenomena of mating types is striking: these too appear, disappear and reappear at nuclear reorganizations; and, further, they are subject to environmental influences at the time of nuclear reorganization.

The fact that environmental conditions at the time of nuclear reorganization influence the relative frequency with which the two mating types are produced might lead one to suppose that the two caryonides from a single reorganized individual would nearly always be of the same mating type, for this is determined by the two macronuclei produced in the same cell at the same time and within 100 micra of each other. Experiments, however, flatly contradict this supposition. As a rule, there is no special tendency for the two macronuclei formed in the same individual to be alike: they are alike only so often as one would draw two red or two black cards from an infinitely large deck that contained red and black cards in the ratio in which the two mating types occur. In other words, the relative frequency of the three possible kinds of results (both caryonides type I, both type II, or one of each type), is determined solely by chance, that is, by the relative fre-

TABLE 8

*P. aurelia*, stocks with mating types I and II. Typical experiments showing that the mating types of the two caryonides from each reorganized individual are independently determined.

Ratio of type I caryonides to type II caryonides	Number of recognized individuals					
	Both caryonides type I		One caryonide type I, one type II		Both caryonides type II	
	Observed	Theoretical	Observed	Theoretical	Observed	Theoretical
2 : 1	42	41.3	40	41.3	11	10.3
1 : 1	35	34.8	70	69.4	34	34.8
1 : 2	22	19.7	76	78.8	79	78.8
1 : 3.4	6	4.5	28	30.9	54	52.6

quency of the two types. In Table 8 appear the observed and theoretically random values for representative diverse mating type ratios: 2:1, 1:1, 1:2, 1:3.4. The agreement between observation and theory is always close.

Ordinarily the same relations are found in variety 3 (mating types V and VI) as in variety 1: the two caryonides produced by a single reorganized individual are no more often alike in mating type than would be expected by chance alone. Nevertheless, in at least one stock (Q) of this variety the two caryonides from each exconjugant are nearly always of the same mating type, providing conjugation and the immediately following nuclear development have taken place at 10° C. Moreover, from the type V and VI individuals that conjugated, there arise two caryonides of type V from the former and two of type VI from the latter: under these conditions, therefore, the mating type ordinarily does not change at conjugation. If, however, the temperature is 20° during conjugation and 10° during nuclear development or 10° during conjugation and 20° during nuclear development, the two caryonides of an exconjugant are alike only so often as expected by chance; only when *both* these processes occur at 10° C. are the caryonides from an exconjugant regularly alike. The temperature at certain periods thus determines not only the mating type ratio, as earlier set forth, but also the *method of inheritance*.

I turn now to those instructive studies of Kimball on inheritance of mating type within the single caryonide. Ordinarily, as Sonneborn (1937) found, and Kimball (1937) confirmed, the mating type is strictly inherited within the caryonide. But Kimball (1939a) found in stock S that when animals of type II undergo uniparental nuclear reorganization and are transformed into type I, the change to type I does not occur at once and occurs at different times in different progeny of the reorganized individual. As a consequence, there are present in a single culture at the same time some individuals of type II and some of type I, so that conjugation can and does occur within such a culture. Kimball demonstrated beautifully



that each pair of conjugating individuals in such a culture consists of one individual of type I and one of type II, in spite of the fact that both are members of the same caryonide. For, if the members of such a pair of conjugants are experimentally separated before fusion has become tight, the one member behaves like type II: it gives the sex reaction when introduced into a culture of type I, not when introduced into a culture of type II; and the other member does just the reverse: it behaves like type I, reacting with type II, not with type I. Kimball further showed that though these individuals are phenotypically different in mating type, they are genotypically the same. For if each is allowed to multiply by fission, the cultures produced by both of them are found to consist exclusively of individuals of type I. The individual that had behaved as type II gave rise to progeny of type I only! In such caryonides, the occurrence of individuals of type II is thus just a briefly transient phase; they are found only among the first individuals produced in the caryonide. As this phenomenon appears only when the caryonide is genotypically type I and arose at nuclear reorganization from ancestors genotypically type II, the ancestral phenotype persists for a short time after the genotype has changed. The same "lag" in change of phenotype following a change of genotype was observed in the inheritance of other characters by Sonneborn and Lynch (1934) and De Garis (1935). From our present point of view, it illustrates that within a caryonide individuals that are all alike in nuclear constitution may transiently differ in phenotype and that individuals of one phenotype may produce descendants of the opposite phenotype in the absence of any further nuclear reorganization. This is a very special though important case and could be discovered only by careful search, for it appears only during the first few days after nuclear reorganization and occurs only when the mating type has been changed from II to I, not when the reverse change from I to II takes place. I have confirmed this observation of Kimball's in other stocks of variety 1 and in variety 3. In the latter, the "lag" occurs when the

mating type changes at nuclear reorganization from VI to V, but not during the reverse change. It occurs at conjugation as well as at uniparental nuclear reorganization; but in some stocks it can not be detected at conjugation, namely, in those stocks showing a period of immaturity, for in these the immature period (7 to 10 days) is longer than the lag period.

In another paper, Kimball (in press) reports a much rarer but even more significant phenomenon. In about 3 per cent. of the caryonides examined in stock S, the mating type changed repeatedly in both directions; animals of type I produced some progeny of type II, for their descendants conjugated among themselves; and animals of type II produced some progeny of type I, for their descendants also conjugated. Both of these changes of type were later reversible. Kimball showed that the two types were actually present both by the fact that conjugation occurred and also by direct tests of mating type on the two individuals of pairs that had been experimentally separated after coming together to start conjugating. He also demonstrated that these repeated changes of type occurred in the absence of new nuclear organizations.

The chief importance of these observations for our general understanding of the phenomena of mating types lies in the fact that these very rare "unstable" caryonides of *P. aurelia* appear to be similar in behavior to the usual type in certain other species. For example, in certain races of *Blepharisma undulans* (Woodruff, 1935) the vegetative progeny of any individual can conjugate among themselves. The question arises therefore as to whether this is to be understood as evidence for the absence of mating types—for the ability of any individual to mate with any other individual. In *P. aurelia*, where such phenomena occur as rare exceptions, Kimball's conclusive analysis shows that conjugation does not occur between any two individuals, but only between individuals differing in mating type. This suggests that similar relations may exist also in species like *Blepharisma*; in these species un-

stable caryonides may be the predominant or only kind present.

(2) *Stocks that contain only one mating type.* In stocks in which only one mating type appears, the genetic phenomena are much simpler. No conjugation occurs *within* such stocks, for only one type is present. When uniparental nuclear reorganization occurs, no change of type takes place. Inheritance is absolute both through vegetative reproduction and nuclear reorganization. The mating type of these stocks is always type I; stocks containing type II only have not been found.

(3) *Hybrids between stocks containing type I only and stocks containing both types I and II, and the progeny of these hybrids.* As will appear, genetic analysis of hybrids between the two kinds of stocks in variety 1 led to the discovery of clear-cut Mendelian inheritance: the first discovery of inheritance in Mendelian ratios in the ciliate Protozoa.

The characters dealt with here are the one-type condition, in which clones remain permanently type I, never changing at nuclear reorganization; and the two-type condition, in which both types I and II appear within a clone. In the latter condition, the two types reproduce true to type only during vegetative reproduction, either mating type yielding caryonides of both types after nuclear reorganization.

From 149 pairs of hybrids between stocks showing these diverse conditions, *all* the caryonides produced by the hybrid exconjugants showed the two-type condition. Hence, the two-type condition is dominant over the one-type condition. If the parent stocks differed in a single pair of genes, the two-type parent stock may be represented as of genotype AA, the one-type parent stock as aa, and the F1 hybrids as Aa.

The hybrids (Aa) were then backcrossed to the recessive parent (aa). Of the resulting pairs of conjugants, half should be of genotype Aa and show the two-type condition and the other half should be of genotype aa and show the one-type condition. In this backcross, 158 pairs of con-

jugants were obtained: in 81 of the conjugant pairs, each exconjugant produced two caryonides showing the two-type condition, and in 77 of the conjugant pairs, each exconjugant gave two caryonides showing the one-type condition. Thus, the backcross yielded the typical Mendelian result for a single gene difference: approximately equal numbers of dominants (81) and recessives (77).

The hybrids ( $Aa$ ) were further tested by interbreeding them to get an  $F_2$  generation composed of 120 pairs of conjugants. In 88 of the pairs each exconjugant gave two caryonides showing the two-type condition and in 32 of the pairs each exconjugant gave two caryonides showing the one-type condition. This is very close to the expected Mendelian ratio of three dominants to one recessive.

Of the  $F_2$  dominants, one-third should be homozygotes ( $AA$ ) and two-thirds heterozygotes ( $Aa$ ). This was tested by crossing nineteen of the  $F_2$  dominants to the recessive parent stock ( $aa$ ). Six of the  $F_2$  dominants crossed to the recessive yielded only dominant progeny; they must, therefore, have been homozygotes ( $AA$ ) and their progeny heterozygotes ( $Aa$ ). The remaining 13  $F_2$  dominants yielded one-half dominants and one-half recessives; they must therefore, have been heterozygotes ( $Aa$ ) and their progeny half heterozygotes ( $Aa$ ) and half homozygous recessives ( $aa$ ). Hence, the  $F_2$  dominants included both homozygotes and heterozygotes in the proportion expected.

The foregoing results supply the strongest genetical evidence that genes and nuclei are exchanged between the two conjugants of a pair. Though this has long been accepted on cytological grounds, it has in recent years been challenged by Diller (1936) and by Wichterman (1937) who hold, on the basis of cytological observations, that exchange of pronuclei between conjugants may not occur in certain species of *Paramecium*. On the genetical side, the common genetic similarity between two individuals that have conjugated together was first discovered by Jennings (1913), whose observations have many times been confirmed, recently, in a striking way by Sonneborn and

Lynch (1934) and by De Garis (1935). The Mendelian analysis set forth above puts this type of observation on a new footing: the genic agreement between the two conjugants of a pair and the relative frequencies of the various gene combinations permit no doubt that in these stocks the nuclei of the conjugants undergo reductions and cross combinations according to the classical scheme.

*Uniparental nuclear reorganization in hybrids.* Turning now to inheritance in hybrids of genotype Aa at uniparental nuclear reorganization, the results are very different from those in either of the parental stocks. When a hybrid exconjugant is allowed to multiply and the progeny undergo uniparental nuclear reorganization, the reorganized individuals are found to yield progeny of two kinds: half of them show the two-type condition and half show the one-type condition. By appropriate breeding tests it was shown that the former are homozygous dominants (AA) and the latter homozygous recessives (aa). In subsequent uniparental reorganizations, no further changes of genotype occur.

How can such results be accounted for? If, at nuclear reorganization, the chromosome number is reduced to the haploid condition and this is followed by an equational division to form two gamete nuclei which fuse, the genetical results observed would occur. Thus, in heterozygotes (Aa), reduction would give in half the animals A and in half a; subsequent formation of gamete nuclei and syn-carya would transform the former half into AA, the latter into aa.

These cytological processes are essentially those held to occur at uniparental nuclear reorganization by Diller (1936). The genetical evidence thus agrees with his cytological account of autogamy. If the non-autogamous endomixis described by Woodruff and Erdmann (1914) had taken place, the heterozygotes should have remained heterozygotes. Thus far, this has not been observed in a single case. However, the observations of Woodruff and Erdmann were carried out mainly, as is now known, on

variety 2, while the preceding analysis was made on variety 1 of *P. aurelia*. Whether genetic evidence of non-autogamous endomixis can be obtained in variety 2 or under other conditions in variety 1 remains to be determined.

The significance of autogamy in the biology of *P. aurelia* is great. It explains why natural stocks are all homozygous after a short time in the laboratory. The heterozygotic condition can endure only during the few weeks that intervene between a cross and the first nuclear reorganization in the hybrids. Thus *P. aurelia*, though a diploid organism, is ordinarily homozygous. There are no hidden recessive genes. For genetical analysis therefore, they are as favorable as haploids.

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**PARAMECIUM BURSARIA: MATING TYPES AND  
GROUPS, MATING BEHAVIOR, SELF-STER-  
ILITY; THEIR DEVELOPMENT  
AND INHERITANCE<sup>1</sup>**

DR. H. S. JENNINGS

As you will see, the situation in *Paramecium bursaria* differs in important ways from that in *Paramecium aurelia*, giving indeed a very different system.

*Paramecium bursaria*, the green *Paramecium*, is somewhat less familiar to many of us than *Paramecium aurelia* or *caudatum*. Yet it occurs commonly and abundantly in our fresh waters.

In this species too we find that members of the same clone ordinarily do not conjugate together, yet if two clones of diverse origin are mixed, we may get the immediate spectacular clumping and pairing that were shown in the demonstrations. The two clones belong to diverse mating types.

This clumping or agglutination is very like that which occurs in *Paramecium aurelia*; and also like that which occurs in mixtures of the two different types of gametes of some of the lower algae. In *Paramecium* the individuals are so large that their behavior in this agglutination can be readily observed. I should like to say a few words about some of the more striking features of the behavior.

When individuals of different mating type (but of the same "group") are mixed, it is to be observed that the individuals are not pulled together or guided toward each other from a distance. They continue to swim about in the usual way. But these movements of many individuals in a limited space soon bring some of them into accidental contact. An individual of one of the types brushes against

<sup>1</sup> Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

one belonging to the other type. Thereupon the two stick together, exactly as if their surfaces were covered with some strong adhesive material. There is no definite reaction, no coordinated movements of the two. Each of the individuals tries (as it were) to continue moving as before, but as they stick together, both are suddenly stopped, or one drags the other against the motion of the latter's cilia; or if the two anterior ends happen to be in the same direction, the two swim forward together.

Any parts of the body that thus come in contact adhere. The two individuals may come in contact by their aboral surfaces or their rear ends or in any irregular way; thereupon they adhere, and begin to move in an irregular manner resultant upon the divergent action of their free cilia. Often one individual drags another backward or sidewise through the water.

Two individuals thus stuck irregularly together flounder about in the crowded drop, and soon come into contact with other individuals of one or the other type. A third individual adheres irregularly to the two, then a fourth, and this continues until large masses are formed, containing twenty to a hundred individuals or more. In these the individuals are in irregular contact, by any parts of the body. In all this it is evident that the coming together of the individuals is the accidental result of their ordinary motions; they adhere when thus accidentally brought together. Until the individuals come into contact there is no indication of stimulation or of a change in behavior.

In the mixtures containing many individuals of the two types, groups are formed with all sorts of irregular attachments. Within three or four minutes after the two types are mixed, large masses of adhering individuals are formed. Such masses may contain hundreds of individuals. The individuals in the clot adhere firmly together by whatever parts of the body are in contact. They do not move freely with relation to each other, being held seemingly by a rigid physical adhesion. By using two types that differ in depth of color or in other marked ways, it can be seen that the adhesion is always between individuals



belonging to the two different types. The large masses contain thus about equal numbers of the two types.

The masses commonly remain firmly united for about half an hour, then begin slowly to break up into smaller masses. Yet masses of considerable size may remain united for two hours or more. As the masses break up, many longitudinal chains are found to have been formed, the individuals (alternately of different types) being united end to end.

Even in the tightly adhering groups the individuals may shift slightly, moving slowly and with seeming difficulty. In this way the relative positions become slowly changed. Some of the individuals come thus in contact by their oral surfaces. There is now apparently some coordinated motion, till the two individuals come into the typical mating position. This is a slow process, requiring usually an hour or more. As the large masses slowly disintegrate, it is found that many of the individuals have become united in pairs.

In *Paramecium bursaria*, as in *Paramecium aurelia*, the clumping and pairing are dependent on the time of day. In most types they take place most strongly about midday and for three or four hours after. About 5 o'clock P.M. or thereabouts the clumping becomes weaker, and by six or seven it has entirely ceased, and the clots earlier formed completely disintegrate. However, the time scheduled for the reactions differs in the different groups: in one group (Group III), the reaction may occur at any hour of the day or night.

We will now return to the mating types themselves. The phenomena that we have described show that in *Paramecium bursaria*, as in *Paramecium aurelia* there are two mating types. Members of a single type do not conjugate together, but when the two are mixed, clumping and conjugation occur. We may call the two mating types that we have discovered A and B.

Now as we test other clones, we find some that conjugate with A, others that conjugate with B. And as we con-

tinue our testing, we come upon a clone that conjugates readily with *either* A or B. We have found a third mating type, C, whose members do not conjugate together, but do conjugate with both A and B.

Continuing to collect and test clones, we find one that conjugates readily with any of the three—with A or with B or with C. We have a fourth mating type, D.

No matter how long we continue to collect and test clones we never find an additional mating type in this set. There are just four and no more. The relations between A, B, C and D are shown in Table 1, in which a plus sign indicates the occurrence of conjugation. The members of any type, as A, do not conjugate together, but do conjugate readily with members of any of the other 3 types. Every clone of this group belongs to one of the four types.

TABLE 1

	A	B	C	D
A	-	+	+	+
B	+	-	+	+
C	+	+	-	+
D	+	+	+	-

But as we continue to collect and test clones from different regions, we find some that do not conjugate with A, B, C or D, but do conjugate together when mixed. These then form a different group. The clone E conjugates with the clone F, though not with A, B, C or D. We continue to collect clones, and find one whose members conjugate with both E and F. We now have three mating types in our second group, E, F and G. We continue in this manner, adding new types in this group until we have eight. And there we stop; no more are to be found. We have one group in which are four mating types, A, B, C and D; another in which there are eight, E to M. In this second group, as in the first, the members of any type do not con-

jugate together, but do conjugate readily with members of any of the other seven types. The relations of the types in this group are shown in Table 2.

TABLE 2

	E	F	G	H	J	K	L	M
E	-	+	+	+	+	+	+	+
F	+	-	+	+	+	+	+	+
G	+	+	-	+	+	+	+	+
H	+	+	+	-	+	+	+	+
J	+	+	+	+	-	+	+	+
K	+	+	+	+	+	-	+	+
L	+	+	+	+	+	+	-	+
M	+	+	+	+	+	+	+	-

Each type includes many diverse clones. As we collect a new clone, we determine to what type it belongs by testing it with members of the known types. Suppose it turns out to belong to Group II. It is tested successively with the eight types of that group. With seven of them it forms pairs. But invariably it selects one of the eight with which it does not react; with which it never clots nor forms pairs. This therefore is the type to which it belongs; it does not mate with members of its own type. As one tests hundreds of clones gathered from many parts of the United States, it appears astonishing that each one selects as it were one type with which it refuses to conjugate, while with all the others of its group it does conjugate. Of the mating type E there are now known 132 diverse clones collected from different parts of the country—from Maryland to California. Of the mating type F, 29 clones are known, and so on.

As we continue tests we come upon another group of clones that do not form pairs with any of the members of Group I nor of Group II, but do form pairs when two of its clones are mixed together. We have therefore found a third independent group. And as we study these, we find that this Group III, like Group I, contains just four mating

types, N, O, P and Q, which show to each other the relations seen in Table 3.

TABLE 3

	N	O	P	Q
N	-	+	+	+
O	+	-	+	+
P	+	+	-	+
Q	+	+	+	-

Thus, so far as investigation has now gone, the species *Paramecium bursaria* consists of three independent groups, the members of any one group not conjugating with the members of the other groups. The constitution of the species is therefore that which is shown in Table 4.

TABLE 4

A	B	.....	E	F	G	H	.....	N	O
C	D		J	K	L	M		P	Q

Group I and Group III each contain four mating types, Group II eight mating types, so that there are in all sixteen diverse mating types. The mating relations of the 16 types are shown in Table 5.

The geographical distribution of these 3 groups and 16 mating types is of interest. The 4 types of Group I have thus far been found only in Maryland and Virginia, but I shall be surprised if they are not ultimately found all over the United States. The eight types of Group II have been found from Cape Cod, Massachusetts, in the East, to California in the West; from East Aurora, New York, in the North, to North Carolina in the South. Group III have been collected on the one hand from near Pineville, North Carolina, on the other hand from about Provincetown, at the tip of Cape Cod, Massachusetts. It would not be surprising if it turns out that all three groups are generally distributed on the North American continent. As to other continents, nothing is known. Possibly other groups and types will be found.

TABLE 5

	I				II								III			
	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q
I	A	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	B	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	C	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
	D	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
II	E	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-
	F	-	-	-	-	+	-	+	+	+	+	+	+	-	-	-
	G	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-
	H	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-
	J	-	-	-	-	+	+	+	+	-	+	+	+	-	-	-
	K	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-
	L	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-
	M	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-
III	N	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	O	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
	P	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
	Q	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-

The three groups seemingly remain quite independent, never intercrossing. Hundreds of tests have been made by intermixing members of the different groups, but no pairing has occurred.

The three groups are not distinguishable by any well-marked morphological characteristics. Within each group, and indeed within each of the 16 mating types there are many different clones, and these clones may differ from each other greatly in size and form. These differences among the clones of even a single type are so much greater than any differences characteristic of the types or groups that the latter are difficult to detect and perhaps do not exist. There are, however, some physiological differences among the groups. Groups I and II show the mating reactions only during certain hours of the day, while in Group III the reactions occur at any hour of day or night.

And here we must introduce one of the exceptional conditions that soften the hard outlines of the system. Ordinarily, as we have said, the members of a single clone do not conjugate together; they all belong to the same mating type. To obtain conjugating pairs we must mix together clones of two diverse mating types.

But in rare instances one comes upon one or more pairs in a culture of a single clone. This is so rare that one may study particular clones for months and see no examples of it. But in the course of time instances of such self-fertilization of a clone accumulate. Individuals are found that adhere to each other in the way earlier described in the clumping of diverse types; such individuals finally pair completely, exchanging nuclei in the usual way, and producing clones of descendants.

Has the single clone before conjugating differentiated into two mating types? This question is to be answered by isolating the two individuals that cling together and determining by tests whether they belong to the same or to diverse mating types.

This was done in the case of a clone that originally belonged to type D of Group I. A considerable number of pairs appeared in this. Tests for the differentiation into diverse types were carried out as follows. Two individuals that adhere in the way that is a preparation for conjugation are separated before they have become really united, and these are tested separately with members of the four mating types. Also, in a considerable number of cases (eighteen in all), the two individuals thus separated were allowed to multiply till each had produced a numerous clone. The two clones were then tested with the four mating types. In every case it was found that the two individuals that had initiated conjugation did indeed belong to diverse mating types. One belonged invariably to type D, the other invariably to type A. The clone was originally of type D. In some way some of its members have become differentiated into type A; the two types then conjugate. As Sonneborn showed in the paper preceding this, in

*Paramecium aurelia* such a differentiation into two types may occur at the time of the nuclear reorganization known as endomixis. In *Paramecium bursaria* endomixis does not occur at regular intervals; it is indeed extremely rare. Erdmann (1927), who studied this matter, could not find endomixis in isolation cultures at all, but believed that she did find evidence of it in mass cultures. It appears probable therefore that these rare cases of self-fertilization of clones are a consequence of the equally rare occurrence of endomixis. To determine this with certainty will be difficult, because of the extreme rarity of both phenomena. But the observations that I have described show that in the cases of self-fertilization of a clone, we are really dealing as usual with the conjugation of individuals that belong to two diverse mating types.

This brings us to the development and determination of the different mating types. How is it determined to what mating type shall belong a particular individual and the clone that it produces by fission?

Here the first question for examination is: What is the relation of the mating type in the descendants to the mating type of the parents? For ordinary fission the answer to this question is very simple. All the descendants by simple fission of an individual are of the same mating type as the parent individual. The mating type is strictly and simply inherited in ordinary fission.

The only apparent exceptions to this are the rare cases of self-fertilization of a clone, just described. In such cases presumably, as we saw, some special nuclear process has occurred in addition to simple fission. But this matter remains to be investigated.

What do we find as to the inheritance of the mating types at conjugation? The two members of a pair of conjugants—which I shall call the two parents—are always of different mating type, as two parents in higher organisms are always of different sex. What is the relation of the mating type in the descendants of the pair to the two types represented by the parents?

In ourselves and other higher organisms only one combination of the mating types is possible; male with female. But in our infusorian with its many different types, many diverse combinations may occur. In Group I, with its four mating types, A, B, C, D, six different crosses are possible, namely,  $A \times B$ ,  $A \times C$ ,  $A \times D$ ,  $B \times C$ ,  $B \times D$  and  $C \times D$ . In Group II with its eight types, the number of diverse possible crosses is  $\frac{1}{2} (7 \times 8)$ , or 28. In Group III, again 6 diverse crosses may occur. Thus the total number of possible different crosses is 40.

Only a few of these 40 crosses have yet been examined. In Group I all the six possible crosses have been made and to some extent studied, so that I shall set forth only the relations found in that group.

The plan of study is as follows. In each pair the two parents are of diverse mating type, for example, A and D. They conjugate, exchanging halves of their micronuclei, then separate. Each is now allowed to divide into two, and these two are cultivated separately, so that each produces a clone. Thus four clones are produced from each pair, two from each ex-conjugant. For each of these four clones the mating type is discovered, so far as possible, by testing with the four known types, A, B, C, D.

The first thing that one learns in such tests is that for a considerable time after conjugation the descendants of the ex-conjugants do not conjugate at all, with any of the mating types. There is a period of immaturity which lasts usually for some months. The different clones descended from union of the same two mating types vary greatly in the length of the period of immaturity. Many of the ex-conjugant clones of Group I, from a conjugation of June 8 last, are still immature, more than six months after the conjugation. Others have become mature at varying intervals. The shortest period observed for the beginning of mating was twenty-two days from the parental conjugation. But for most clones immaturity lasts for months.

Complete maturity does not come on suddenly. There is a long period of partial immaturity, of "adolescence" in



which the mating tendency is weak and but few individuals of the clone conjugate. In a test mixture of a hundred or more individuals from each of two clones, frequently but one or two pairs will be formed, and these only after the two clones have been together for several days. This makes much difficulty in determining the mating type of young clones. In Group I, the mating type has thus far been determined by test for 250 young clones, derived from 164 ex-conjugants, belonging to 116 different pairs.

A third thing discovered in the tests is a matter of much interest. We discover that at first the young clones differentiate into two types only, not into the final four. One of these two types conjugates with the adult types C and D, but not with types A nor B. On our usual principles it may be called the type AB. The other type conjugates with the adult types A and B, but not with C nor D. It will have to be called the type CD. Thus in some of the pairs at a certain period all the four clones descended from the pair are of the type AB; in others all the four clones from a pair are of the type CD.

These two types AB and CD differentiate farther in the course of time, so as to form the four adult types A, B, C and D. That is, some of the individuals of the type AB, which at first mate only with C and D, in time acquire the power to mate also with type B, but not with type A. These have therefore developed from type AB to type A. Those from some other pairs develop into type B. In the same way the clones of type CD derived from some pairs develop into type C, while those from other pairs develop into type D.

Thus the four adult types A, B, C, D are slowly developed from the immature individuals, which first differentiate into two types; then each of these two develops into two further types. The types A and B are more closely related to each other than either of them are to C or to D. A and B are closely related, also C and D are closely related.

The offspring have therefore after some weeks or months reached the adult condition, in which there are four

diverse mating types, A, B, C, D. We may now compare the final types in the offspring with the types in the parents. First, as to general relations: Table 6 shows those

TABLE 6

Group I				
Four Types: A, B, C, D				
Six Possible Crosses: $A \times B$ , $A \times C$ , $A \times D$ , $B \times C$ , $B \times D$ , $C \times D$				
Mating Types Given Thus Far by the Six Crosses (Conjugation of June 8, 1938, or Earlier):				
<i>Cross</i>	<i>yields</i>	A, B,	C,	D
$A \times C$		A, B,	C,	D
$A \times D$	"	A, B,	C,	D
$B \times C$	"	A, B,	C,	(D?) (Many immature)
$B \times D$	"	A, B,	(C?),	D (Many immature)
$A \times B$	"	A, B		(Most still immature)
$C \times D$	"	C, D		(Most still immature)

thus far observed. A certain cross of two parents, as  $A \times C$ , gives rise to all the four types A, B, C, D. This is true also for the cross  $A \times D$ ; and probably also for two others,  $B \times C$  and  $B \times D$ , though these are not fully worked out yet, most of the clones being still immature. The remaining two crosses are those between the two closely related types,  $A \times B$  and  $C \times D$ . Each of these pairs of types were, as you remember, originally united in one type AB or CD. The crosses of these two related types have thus far given only the two parental types. But the data are incomplete; most of the young clones from these crosses are still immature.

Coming now to specific and numerical relations between the parental types and those of the descendants, we discover the relations shown in Table 7, which gives the results for the crossing of type A with type D.

TABLE 7

CROSS, TYPE A BY TYPE D. 31 PAIRS: FOUR CLONES FROM EACH PAIR, TWO FROM PARENT A, TWO FROM PARENT D

		From parent A	From parent D
13 pairs gave	.....	A, A	A, A
14 " "	.....	D, D	D, D
2 " "	.....	B, B	B, B
2 " "	.....	C, C	C, C

The first important fact to be observed is that in almost

all cases—in *all* cases in this cross  $A \times D$ —the four clones derived from a given pair are alike in their mating type; all four are A, or all are D, or all are B or C.

And second we find that in the great majority of cases the type to which the four belong is the same as that of one of the two parents. In the cross of  $A \times D$ , shown in Table 7, 27 of the pairs gave descendants that were all like one of the two parents—like either A or D, while four did not. About half the pairs give descendant clones that belong to one of the parental types, the other half to clones that belong to the other parental type. In the case of the cross just mentioned, there were 31 pairs from parents that were A and D. Of the 31, thirteen gave descendants that were all of type A, while fourteen gave descendants that were all of type D.

A third important fact is shown in Table 7. A few of the pairs—four out of thirty-one in this case—give descendants that are not of either parental type. Two give clones that are all of type B, two others clones that are all of type C.

These relations appear typical for most of the crosses in Group I. Of the 250 ex-conjugant clones fully tested, 205, or 82 per cent., belong to one of the two parental types, while 45, or 18 per cent., are unlike the parents in type.

How can these relations be accounted for? Leaving out of account for the present the few exceptional cases, the reappearance of the two parental types in equal numbers in most of the descendants may be accounted for by conditions similar to those which result in the production of equal numbers of the two sexes in higher organisms. Suppose that the parental types are diverse in a single chromosome or gene, so that one type is XX while the other is XY. Then by their union we shall get again in the next generation equal numbers of XX and XY; that is, the two parental types.

But why should all the descendants of a particular pair be alike in their mating type? This is much as if all the

members of a family of children should be alike in sex: all males or all females.

This seems to result from certain special conditions that exist in the ciliate infusoria. During conjugation the micronucleus of each ex-conjugant divides twice, yielding four pronuclei. Three of these disappear, leaving but one. This divides again—the so-called third division—and one of the two products is exchanged for one from the other member of the pair.

If the nucleus of one individual contains XX, while that of the other contains XY, then if reduction occurs at either the first or second division, the four nuclei from the parent XX will be X, X, X, X, while from the parent XY the four will be X, X, Y, Y. Now three of the four disappear in each case, leaving but one. In the parents XX will remain the single nucleus X, while in the cases of parents XY there will be left in half the cases the single nucleus X, in the remaining cases the single nucleus Y.

Notice first the cases in which each of the two parents now contains the single nucleus of constitution X. Next occurs the third division of the nucleus, after which each of the two parents obviously contains two pronuclei, X, X. They exchange one of these, after which it is evident that each parent contains the nuclear combination XX. The two parents are therefore alike, both being of the type XX; their descendants by vegetative fission will therefore all belong to the single type XX.

In the other possible case, after reduction at the first or second division with disappearance of three of the four nuclei, one parent contains the single nucleus X the other the single nucleus Y. After the third division one has two pronuclei X, X, the other two pronuclei Y, Y. Now occurs the exchange of one pronucleus, after which each of the two parents obviously contains the combination X, Y. Again the two parents are alike and both are of the type XY; their vegetative descendants are therefore all of the single type XY.

If however reduction did not occur until the third nuclear

division, the parent XY would contain two final pronuclei, one of which would be X, the other Y. The result would be that one of the two ex-conjugants would receive the combination XX, and all its descendants would be of that type, while the other ex-conjugant of the same pair would contain XY, and all its descendants would be of that type. The descendants of the two ex-conjugants of such a pair would be of diverse mating types. The fact that in almost all cases they are of the same mating type is perhaps strong evidence that reduction usually occurs at the first or second division of the micronucleus, not at the third division.

But in a few cases, the descendants of a single pair are not all alike in type. Among forty-eight pairs in which the type of the descendants of both ex-conjugants was determined, there were six pairs in which two different types occurred among the descendants. The mating types of the descendants of these six pairs are shown in Table 8.

TABLE 8

PARAMECIUM BURSARIA. SIX PAIRS (OUT OF 48) THAT GAVE DIVERSE MATING TYPES AMONG THE FOUR CLONES DERIVED FROM EACH PAIR

Parents	Mating types of the descendants	
	from Ex-conjugant 1	from Ex-conjugant 2
A × C .....	A, B	B, B
A × C .....	D, B	D, D
A × C .....	A, B	?, ?
A × C .....	A, ?	C, ?
B × D .....	B, B	A, ?
C × (B?) .....	C, C	B, B

In three of the six pairs (the last three of the table) the descendants of the two ex-conjugants differed in type. This is the situation that would be brought about if in these three cases reduction had occurred exceptionally at the third division of the micronucleus instead of earlier.

But in three other cases, the descendants of a single one of the ex-conjugants included two types. One of the two clones resulting from the first division after conjugation was of one type, the other of a different type. In rare cases therefore the diverse mating types are segregated at the first division of the ex-conjugant.

There remain the cases in which the descendants of a

pair are all of a different type from either of the parents. As shown earlier, there were four such pairs from parents that were type A by type D. Two of these pairs gave 4 clones that were all of type B, the other two, four clones that were all of type C.

In considering such cases, it is to be remembered that the type B is derived with the type A from the single young type  $\overline{AB}$ , while type C is derived with type D for the single young type  $\overline{CD}$ . What happens then is this. When the parents are  $A \times D$ , all the young descendants of about half the pairs are of the type  $\overline{AB}$ , while the young descendants of the rest of the pairs are all of the type  $\overline{CD}$ . Then most of the  $\overline{AB}$  develop into the parental type A, while a few develop into B. Similarly, most of those that are of type  $\overline{CD}$  develop into the parental type D, while a few develop into the non-parental type C. The nature of the underlying factors and influence for these changes are as yet unknown.

The conditions that I have illustrated for the results of the cross  $A \times D$  appear typical, so far as examination has gone, for the other crosses in Group I: but much remains here to be discovered.

And now a few words in conclusion as to the relation of the phenomena described to those found elsewhere in the world of organisms.

As you have seen, the conditions in *Paramecium bursaria* differ from those in *Paramecium aurelia* in the fact that in any race or group of aurelia there are but two mating types, so that the situation there resembles that in the higher animals and plants, with their two sexes. In *Paramecium bursaria*, on the other hand, each group contains four or eight mating types. The development shows, as I have already set forth, that at first there are but two types, and that each of these differentiates into two, making four. The fact that in Group II there are eight types suggests that in this group differentiation has gone one step farther, each of the four types differentiating into two to make

eight. All this suggests that the condition with but two types is the original one, from which the condition with multiple types has developed.

In the flagellates, according to the magnificent investigations of Moewus, there are in each species or race, as in the races of *Paramecium aurelia*, just two mating types. The flagellates are haploid, so that the cells of the two mating types are closely comparable to the male and female gametes of higher organisms. Moewus therefore considers the phenomena in the flagellates as strictly sexual, speaking of the two types as the two sexes. In the ciliates of course the organisms are diploid, so that the different mating types are not directly comparable to male and female gametes, but rather to the two types of somatic individuals that constitute the two sexes in higher organisms. Yet the situation in the ciliates differs from that in the typical bisexual organisms in the fact that in most of them both members of the pair produce descendants.

The many diverse mating types in *Paramecium bursaria* invite comparison with the facts of "multipolar sexuality" in certain fungi. Yet the differences between the cases are greater than their resemblances. The sexually reacting parts in the fungi are haploid, not diploid. Further, in the fungi the situation is such that usually a given mating type or sex mates with only one other type out of four, whereas in *Paramecium bursaria* each mating type mates readily with any of the other types of its group. The conditions in the fungi differ in principle and in details from those found in the infusorian.

The phenomena in *Paramecium bursaria* appear most directly comparable with the so-called "self-sterility" of some of the higher plants and animals. The single clone of the infusorian is comparable to the single self-sterile plant, it ordinarily does not fertilize itself; its cells do not unite in conjugation. But the single clone, like the single plant, may be fertilized by another; the cells of the single clone unite in conjugation with those of other clones, giving viable descendants.

But this is by no means the whole story, either in the infusorian or in the self-sterile plant. The further phenomena in the two cases show many parallels in their details. In some cases the self-sterile plant becomes at times self-fertile, giving viable offspring; this is true also, as set forth earlier, for the single clone of the infusorian. Again, the phenomenon is not strictly one of "self"-sterility merely, in either the plant or the infusorian. The single plant is sterile also with certain other plants, just as the single clone of *Paramecium* is sterile with certain other clones (those belonging to the same mating type). In most self-sterile plants the individuals can be divided into classes, such that those belonging to the same class are infertile together, while those belonging to diverse classes are fertile together. Such classes are comparable to the different mating types of *Paramecium bursaria*. In this infusorian the number of such types is definite and limited: there are four in each of Groups I and III, eight in Group II; while in *Paramecium aurelia*, as Sonneborn shows, there are but two in each group. The conditions in the infusoria emphasize the similarity and possible relationship between the phenomena of self-sterility and those of sexuality.

For the division of the infusoria species into groups that do not cross-conjugate, I have not found parallels in the accounts of self-sterility in higher organisms. Its real parallel appears to be the actual differentiation of a genus into species that do not cross. The groups may perhaps be considered slightly marked cases of differentiation into incipient species: possibly it will eventually appear best to give them different specific names.



# STUDIES ON CONJUGATION IN *PARAMECIUM MULTIMICRONUCLEATUM*<sup>1</sup>

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MAUPAS (1889) in his classical studies on conjugation of the protozoa showed that conjugation was dependent upon both internal and external conditions, but clear evidence of the nature of the internal factors came only recently with the conclusive studies of Sonneborn (1937, 1938) on *Paramecium aurelia* and Jennings (1938) on *Paramecium bursaria*, in which mating types were described for these two species. With a better understanding of the internal conditions, the effect of the external conditions on conjugation can be more effectively studied. It is probable that a solution of many of the problems of conjugation lies in a proper understanding of the interrelationships of both intrinsic and extrinsic factors. In this paper are recorded some experiments on the action of internal and external factors in the conjugation of *Paramecium multimicronucleatum* Powers and Mitchell.

## STUDIES ON INTERNAL FACTORS

In experiments recently reported (Giese and Arkoosh, 1939) eight local stocks of *P. multimicronucleatum* were tested for presence of mating types. In seven of the stocks conjugation occurred among the progeny of a single individual. Stained samples revealed no endomixis during the period of divisions preceding conjugation, and it is known that endomixis occurs relatively infrequently in this species of *Paramecium* (Stranghøner, 1932). Therefore the animals should be of similar nuclear and genetic constitution. To test if differentiation occurs just preceding

<sup>1</sup> Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

union, animals were separated from one another when in incipient conjugation and cultures were grown from each of the animals of the pair. Progeny of each of the two showed no greater attraction for one another than for their own kind. Progeny of ex-conjugants showed the same behavior. The experiments are described in detail in the paper cited.

That the stocks tested are relatively constant in behavior with respect to mating character is indicated by the fact that one of the stocks was cultured for over five years without variation from the pattern whenever tested. Others have been tested for shorter periods of time but adequately to indicate that the behavior is not aberrant and temporary.

The eighth stock, however, showed no conjugation when tested in the manner used for the other stocks. This exceptional stock (number 2) was subjected to variations in environmental conditions which according to various authors are conducive to conjugation. At the end of a series of several hundred negative experiments conjugants appeared, and thereafter occurred regularly in mass cultures under the conditions conducive for conjugation in the other stocks (Giese, 1938a). Isolation cultures now demonstrated the presence of two types in this population—one (2C) in which progeny of a single individual did not conjugate with one another regardless of conditions, another (2E) in which progeny of a single individual conjugated with one another. But most interesting was the fact that individuals of 2C readily conjugated with individuals of 2E provided both were in the proper physiological state. Thus when one individual of 2E was introduced into a culture of 2C, immediate clumping occurred and a conjugating pair appeared later. When five animals of 2E were added to 2C, not more than five pairs appeared. When 50 of each, 2C and 2E, were mixed as many as 80 or more of the 100 conjugated.

As pointed out above, progeny of a single member of 2E conjugated among themselves but not as readily as with 2C, particularly when the number of generations subse-

quent to subculture was small. When individuals of 2E in incipient conjugation were separated from one another and cultures were grown from each animal, members of the subcultures were found to attract one another more intensely than do members of a given culture (Table 3, Giese and Arkoosh, 1939). This indicates a secondary segregation of mating types in this stock.

In stock 2 the excretion crystals present are larger than those of other stocks, therefore in mixtures members of this stock can be distinguished from other stocks. Attempts were made to determine if mixed mating occurs between stock 2E or 2C and each of the other seven stocks. While conditions were apparently optimal and conjugation occurred, matings between stock 2 and the other stocks was not observed, each individual mating with its own kind. This points to (1) the presence of several sets of mating types, or (2) a situation not comparable to that in *P. aurelia* or *P. bursaria*.

One may conclude from these experiments that (1) either mating type differentiation is lacking in some stocks of this species; or (2) in such stocks mating types differentiate during vegetative division; or (3) reversal of mating types occurs during vegetative division. Kimball (1938) has recently shown that the second case occurs rarely in *P. aurelia*. When adequate means for testing the phenomena are established in *P. multimicronucleatum* it should be possible to decide between these alternatives. It is possible that the situation which is the exception in *P. aurelia* is the rule in *P. multimicronucleatum* and that in the latter species clearly defined mating types are the exception. More data are necessary and experiments are in progress on a number of stocks from various other localities.<sup>2</sup>

Aside from mating type differentiation another internal factor—the “maturity” of a stock—conditions conjugation. Thus in some of the stocks of *P. multimicronucleatum* a certain time must elapse subsequent to conjugation before

<sup>2</sup> Since this paper was written well defined mating types have been found in both local and distant stocks.

mating is again possible. It is well known that ex-conjugants of many species of protozoa show low viability. Experiments on some stocks of *P. multimicronucleatum* have shown that conditions optimal for growth of vegetative individuals are often deleterious for ex-conjugants and that survival may be increased by modification of the conditions (*e.g.*, decrease in food concentration and culture temperature). "Maturity" may be bound up with the recovery of a "proper" physiological state.

#### STUDIES ON ENVIRONMENTAL FACTORS

While proper internal conditions are essential for conjugation, mating occurs only under certain environmental conditions and the environment modifies the time of onset, the intensity and the duration of the epidemic of conjugation. The factors which have most often been considered as important are food and its correlatives, temperature, salts (type and concentration), pH, metabolites and population density.

*Food* has been considered the most important single factor. In mass cultures conjugation occurs when conditions have become somewhat adverse for multiplication. Maupas (1889) surmised that lack of food was a necessary condition for conjugation. Jennings (1910) reported that it was rather the lack of food after a period of plenty. Many other conditions are changing along with the change in food, and Bělař (1924), using *Actinophrys sol*, offered the first conclusive proof that decline in food after a period of plenty was indeed the most important factor. Similar experiments with the same results have been performed here with a number of stocks of *P. multimicronucleatum* and *P. caudatum* (Giese, 1935, 1938a, and Giese and Arkoosh, 1939).<sup>3</sup>

<sup>3</sup> Baitsell (1912) observed that conjugation occurred in some substrates, not in others. In experiments carried out here on *P. multimicronucleatum* (stock 1 was used) substrate was found to be of no consequence except that in more nutritious substrate conjugation was proportionally delayed, but equally good epidemics occurred in lettuce, hay, wheat, mushroom, apple and rice infusions.

*Temperature* affects the time of onset, the intensity and the duration of an epidemic of conjugation. Conjugation occurred between 8.4° and 30° C., but the number in union at these extremes was very small. Animals grown and kept at 15°, 20° and 26° C. will conjugate when the food is depleted, the conjugants appearing soonest at the highest temperature. A temperature change is thus not necessary for conjugation, yet in most cases a change from 26° C. to 20° C. was followed by an epidemic of greater intensity (65 per cent.). This was true especially for animals of stocks I and II which showed relatively few conjugants when grown and kept at 26° C. Placed at 15° C., however, the number of conjugants was smaller than at 20° C. Stocks 2E, 6, 7 and 9 showed good epidemics (up to 60 per cent.) even when grown and kept at 26° C. Animals of all stocks grown and kept at 30° C. seldom conjugate, but when they are placed at lower temperatures at the appropriate time, conjugation epidemics occur.

When animals which were showing the mating reactions at 20° C. were subjected to 8.4° C., they disbanded almost completely, union being rare. When such a culture was returned to 20° or 26° C., the mating reactions began again even when twelve or more hours intervened. In some instances this was repeated a second time. The reacting state is apparently maintained for a long time at this lower temperature, but the reactions can not be completed until the temperature rises to a suitable level. Subjection of cultures to 5.4° C. acted similarly when it was not lethal. When animals in the reacting state are subjected to 30° C., they rapidly lost the tendency, indicating a reaction with a fairly high temperature coefficient.

To determine the effect of different temperatures upon duration of epidemics of conjugation, conjugants of 2E were picked from a culture when a maximum were in conjugation and were suspended in culture fluid at 15, 20 and 26° C. The results, plotted in Fig. 1, show that the duration of the epidemic is directly dependent upon the temperature.

*Salts* of a specific type and in certain ranges of concentration have been considered as necessary for conjugation by Enriques (1909) and Zweibaum (1912). With stocks of *P. multimicronucleatum* used here this is apparently not the case. It is true that paramecia washed and suspended in distilled water conjugated but rarely and that too high

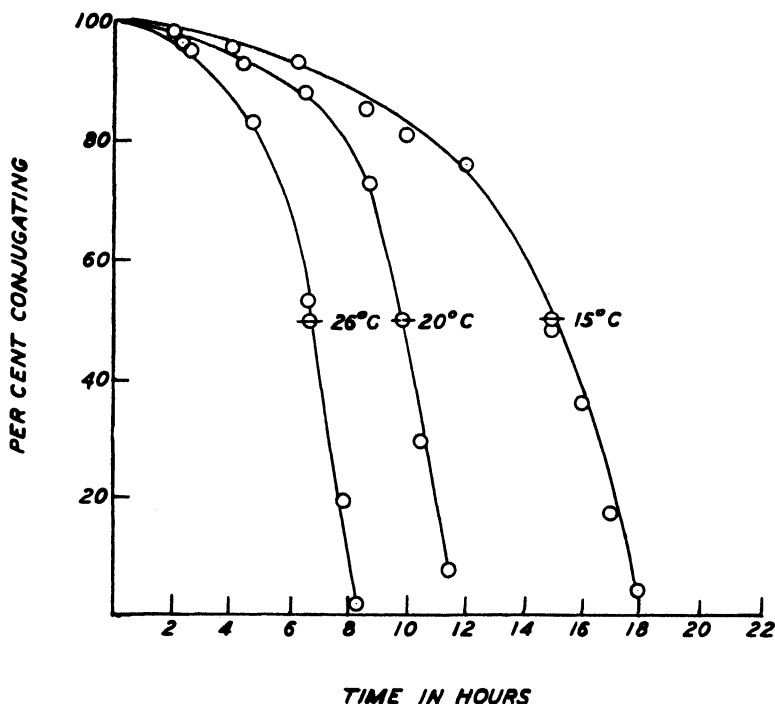


FIG. 1. Rate of ex-conjugation at different temperatures. Conjugants (stock 2E) were removed when the epidemic was at a maximum. Each curve is the average for 800 animals.

a concentration (0.2 per cent. balanced salt solution)<sup>4</sup> also prevents conjugation. However, the culture medium (such as lettuce or hay infusion) can be replaced by balanced salt solutions of a considerable range of concentration (0.15 to 0.003 per cent.) without preventing epidemics of conjugation. Furthermore, addition of  $\text{FeCl}_3$  or  $\text{AlCl}_3$ ,

<sup>4</sup> See Giese, 1938a, for a description of the balanced salt solution used here. A 0.3 per cent. (total salt) solution of this medium was lethal to the paramecia (stock 1).

claimed to be so effective by Zweibaum for increasing intensity of conjugation epidemics in *P. caudatum*, had no significant effect on *P. multimicronucleatum*. Thus cultures in balanced salt solutions buffered at pH 7.0 made up to 0.0000166M.  $\text{FeCl}_3$ , showed 69.0 per cent. (with a standard deviation of  $\pm 9.1$  per cent.) in conjugation, the controls,  $60.0 \pm 7.5$  per cent. Other cultures suspended in  $\text{AlCl}_3$  solutions of six different concentrations (between 0.000416M and 0.00000208M made up in balanced medium buffered at pH 7.0) showed epidemics about equivalent to controls and, in all, averaged  $62.1 \pm 5.8$  per cent., the controls,  $61.0 \pm 12.3$  per cent. And finally, suspension in pure salt solutions<sup>5</sup> of  $\text{NaCl}$ ,  $\text{MgSO}_4$  and  $\text{CaCl}_2$  (each 0.012 per cent.) reduced the intensity of the epidemic to between 20 and 40 per cent. of the population but did not prevent conjugation.

To determine if the action of the salts in the medium is mainly osmotic, the paramecia were washed and suspended in 0.007M sucrose (approximately equivalent to the ionic molarity of the balanced salt solution buffered at pH 7.0)<sup>6</sup> In all cases paramecia conjugated comparably to controls. Whatever the other functions may be, the function of the salts is apparently mainly to maintain the osmotic relations, since most of the salt can be replaced by sucrose.

That the duration of an epidemic of conjugation is also not greatly influenced by the salt content of the medium is indicated by experiments summarized in Fig. 2. Ex-conjugation occurs at about the same rate in buffered and unbuffered salt solutions, in sucrose solution and in culture medium.

A change in *hydrogen ion concentration* affects many biological processes, therefore tests were made to determine what effect a variation in pH had upon conjugation.

<sup>5</sup> Dilution of original medium 80,000; 60,000; and 10,000 fold in the three cases, respectively.

<sup>6</sup> Dilution of salt medium 1,500–1,800 fold in these experiments. Similar experiments on paramecia with 0.012 per cent. urea gave similar results. Experiments with *Blepharisma undulans*, using sucrose, also gave similar results.

Little difference was observed in the intensity of conjugation for the pH range studied, those at pH 6.0 showed  $58.1 \pm 6.7$  per cent. in conjugation; at 7.0,  $59.0 \pm 8.6$  per cent.; at 8.0,  $65.5 \pm 6.0$  per cent.<sup>7</sup> In Fig. 2 it is observed that this pH range also has but little effect upon the duration of conjugation.

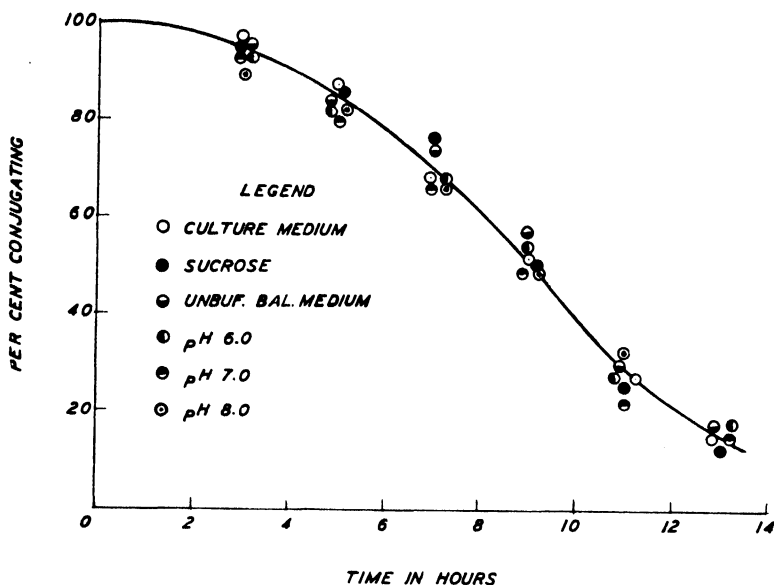


FIG. 2. Rate of ex-conjugation at 20° C. of conjugants (stock 7) subjected to various solutions when at the height of an epidemic. The curve is the average for 300 animals in each solution.

*Crowding* a flourishing culture is a favorite way of producing an epidemic of conjugation. Crowding might act in one of several ways: by (1) increasing the concentration of metabolites; (2) increasing the chance for contact between individuals; (3) giving a greater chance for selection of suitable mates; (4) bringing about a quicker exhaustion of the food.

When animals are washed free of food in salt solutions and one group is concentrated twenty times as much as the other, conjugation occurs at approximately the same time

<sup>7</sup> Solutions and buffers made up as described in Giese, 1938a. Stocks 1, 6 and 7 tested.



in the two cultures. But if both are fed a given quantity of bacterial suspension, conjugation occurs much sooner in the concentrated culture, the time difference depending on the quantity of bacteria added. There is thus no correlation with metabolites which are more concentrated where the paramecia are more numerous but a direct correlation with exhaustion of food.<sup>8</sup>

To test the third possibility—whether selection of mates was facilitated by larger numbers—incipient mating pairs of stock 7 were isolated in hanging drops in moist chambers and in only 21 per cent. of the drops did conjugation occur. When four paramecia were present in a drop, conjugation occurred in 49 per cent. of the cases. When 12 paramecia were present in a drop, conjugants appeared in every drop. In a series in which an average of 44 animals were present per drop, 39.4 per cent. of the total population conjugated (in some extreme cases 84 per cent. of the population of a given drop conjugated.<sup>9</sup> In all experiments contact was readily established and the pairs swam together for some time before separating. It is readily seen from the above that contact is not lacking, yet some animals seem to find their mates unsuitable. It is known that large animals tend to mate with large, small with small (Jennings, 1911), although there are exceptions to the rule (Giese, 1938b). Other factors at present less obvious than size may play a part in selection as well.

#### DISCUSSION

Experiments have demonstrated mating types stable for a given caryonide in only one of eight local stocks of *P.*

<sup>8</sup> Metabolites of various bacteria also seem neither to accelerate nor to retard conjugation. Paramecia (stock 1) suspended in Seitz-filtered infusions of *Pseudomonas fluorescens*, *Bacillus subtilis*, *B. prodigiosus*, *B. pyosepticus* and various mixed cultures conjugated as well as controls.

<sup>9</sup> Even more striking results were obtained with a stock (No. 12) of *P. caudatum* showing clear-cut mating types. When two individuals were present, of 151 cases, 35.8 per cent. conjugated; when four paramecia were in a drop, conjugants appeared in 71.5 per cent. of the drops (35 cases); when six paramecia were in a drop (5 of one mating type, one of the other), conjugation occurred in 85.5 per cent. of the drops (68 cases).

*multimicronucleatum*. That this finding is not peculiar to the local stocks only is indicated by the fact that Gilman (1938) has made similar observations on stocks of this species collected in the vicinity of Johns Hopkins University. It is not impossible that in *P. multimicronucleatum* the evolution of such mating types is only beginning. More extensive experimental data are, however, necessary to show whether this is the case or whether evolution has gone on in a direction quite different from that in *P. aurelia* or *P. bursaria*.

Both internal and external factors influence the conjugation of *P. multimicronucleatum*. Thus after conjugation paramecia of some of the stocks must achieve an internal state of "maturity" before another conjugation. Also paramecia of race 2C will not conjugate in the absence of 2E; other stocks appear to be self-fertile. But in addition to the satisfaction of the above internal conditions, the animals must be subjected to certain external influences before conjugation will occur.

While many environmental factors modify the onset, intensity and duration of an epidemic of conjugation, in many cases the action is mainly if not entirely by their effect upon the food available and the nutritive state of the protozoans. Thus if paramecia are grown at different temperatures they conjugate soonest at the highest temperature, but in each case when the food supply is beginning to be depleted. Also washing the paramecia free of food in appropriate salt solutions facilitates conjugation. And crowding paramecia in ordinary infusions favors conjugation in part by bringing about a quicker exhaustion of food. Depletion of the food leads to the development of an internal nutritive state conducive to conjugation.

This nutritive state favorable to conjugation varies in different species. Thus Sonneborn (1938) observed that in certain stocks of *P. aurelia* conjugants appear even while the animals are still very vigorous and even contain food vacuoles. Jennings (1938) found that a certain amount of starvation was necessary for *P. bursaria*. Zwei-

baum (1912) reported that considerable starvation was necessary for *P. caudatum*. Of the stocks of *P. multimicro-nucleatum* used here, stocks 1 and 11 required more starvation than the others, and even when mating types were present as in stock 2, a certain degree of starvation was also found to be essential, as discussed elsewhere (Giese and Arkoosh, 1939).

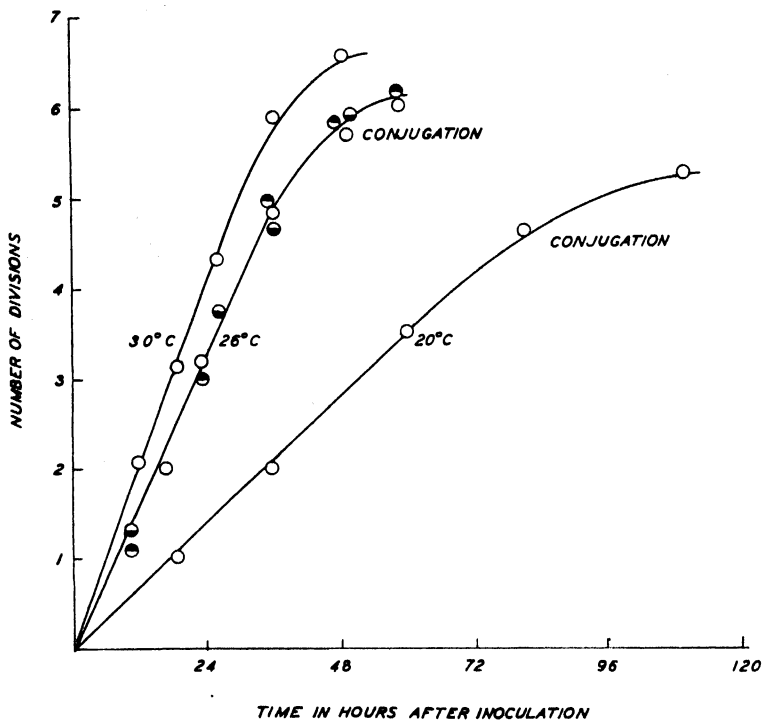


FIG. 3. Appearance of conjugants at different temperatures. Each point is the average of 10 cultures (stock 7).

While the exact state favorable to conjugation varies for different stocks of a species and for different species, in all cases there is one common denominator—a change from the active growth and multiplication characteristic of the vegetative state to a state of slower growth and multiplication. For animals ready to conjugate are growing less actively, have become reduced in size (Jennings, 1911) and in some cases are consuming less oxygen (Zweibaum,

1921). Conjugants are observed in a population only after an inflection in the growth rate curve (Fig. 3), or in the extreme cases after the division rate is zero.

That it is the relative change in division and growth rate, not an absolute change, is shown by the fact that the absolute rate of multiplication is different at 20, 26 and 30° C., yet as illustrated in Fig. 3, in each case conjugation follows a decrease from the division rate characteristic of each temperature (at 30° C. conjugation occurs only if the paramecia are placed at the proper time at a lower temperature). Conjugants are smaller than non-conjugants or vegetative individuals. The size reached before conjugation occurs, *i.e.*, the decline in nutritive state, preceding conjugation is also relative, as was demonstrated for *Blepharisma* (Giese, 1938b) and depends upon the preceding nutritional history of the animal. The relative change in nutrition seems to set going a chain of reactions which lead to the "sticky state" characteristic of the animals in incipient conjugation.

That the changes in appearance, activities and nutrition preceding conjugation are superficial outward signs of a more fundamental change in internal state is granted, and it is the search for the nature of this fundamental change that invites one to further research.

#### SUMMARY

(1) Experiments on *Paramecium multimicronucleatum* indicate that mating types occur, although most of the stocks examined exhibit conjugation among progeny of a single individual.

(2) Environmental factors modify the time of onset, the intensity and duration of an epidemic of conjugation, and, when variation from the usual condition is extreme, may prevent conjugation.

(3) Some environmental factors, if varied within relatively small limits, have very little influence on the epidemic of conjugation, *e.g.*, pH, salts and metabolites.

(4) Other factors of the environment, such as tempera-

ture and crowding of the paramecia, may modify the time of onset, the intensity and duration of an epidemic of conjugation, partly by direct effects, partly by effects upon the rate of exhaustion of the food.

(5) A relative decline in nutrition precedes conjugation in all stocks, whether they show selfing or mating types.

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# MATING TYPES IN *PARAMECIUM CAUDATUM*<sup>1</sup>

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THE following account of mating types in *Paramecium caudatum* is a preliminary report of investigations on the number and interrelations of the types, on the conditions required for the mating reaction and conjugation, and on the inheritance of type within the clone.

The material examined consists of cultures derived from individuals collected from ten different natural habitats: seven in or near Baltimore; two near Woods Hole, Mass.; and one in Connecticut. From each collection one to six animals were isolated and cultures grown from each. In addition, in those collections in which conjugation took place, pairs that had not yet firmly united were separated and cultures grown from each member of the pair. Since presumably the animals starting to conjugate were of different mating types the cultivation of split pairs greatly increased the chances of obtaining cultures of opposite mating type from the same natural source.

In order to discover whether mating types occurred among these clones and, if so, how many there were and how they were interrelated, samples from each clone were mixed, in twos, with samples from each of the other clones and the mixtures were examined for conjugants. When only small cultures were available about fifty animals from each culture to be tested were used in each test. Since in these small cultures the animals were not, as a rule, in the proper condition to give an immediate reaction they were placed in four drops of culture fluid in order that they might be in a position to conjugate when they reached the proper condition. The mixtures were placed at 24° C.

<sup>1</sup> Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

and examined for conjugants after twenty-four hours. As controls, one hundred animals of each clone were kept unmixed but otherwise treated in the same manner as the mixtures. When conjugation occurred in the mixture but not in the controls the two cultures mixed were regarded as being of opposite mating types. If larger cultures, in which the animals were in reactive condition and aggregated in a dense band on the side or bottom of the container, were available, larger numbers were mixed and the mixtures examined immediately for the characteristic rating reaction, clumping of the paramecia.

The results of these clonal mixtures are given in Table 1

TABLE 1

INTERACTION OF CLONES OF *PARAMECIUM CAUDATUM* ISOLATED FROM NATURAL COLLECTIONS. THE LETTER GIVES THE SOURCE OF THE CULTURE, THE ROMAN NUMERALS THE MATING TYPE. CONJUGATION IS INDICATED BY +, LACK OF CONJUGATION BY -

	CI	DI	CII	DII	MIH	PIH	WHI	EIV	MIV	UIV	WIV
CI .....	-	-	+	+	-	-	-	-	-	-	-
DI .....	-	-	+	+	-	-	-	-	-	-	-
CII .....	+	+	-	-	-	-	-	-	-	-	-
DII .....	+	+	-	-	-	-	-	-	-	-	-
MIH .....	-	-	-	-	-	-	-	+	+	+	+
PIH .....	-	-	-	-	-	-	-	+	+	+	+
WHI .....	-	-	-	-	-	-	-	+	+	+	+
EIV .....	-	-	-	-	+	+	+	-	-	-	-
MIV .....	-	-	-	-	+	+	+	-	-	-	-
UIV .....	-	-	-	-	+	+	+	-	-	-	-
WIV .....	-	-	-	-	+	+	+	-	-	-	-

where + signifies that conjugation occurred in the mixture of the clones represented on the corresponding row and file, and - signifies that it did not. A blank signifies that the mixture was not tried. Clones collected from the same source in nature are represented by the same letter. As shown in the table the clones in collections C and D form one interbreeding group, those in collections E, M, P, U and W form another; the two groups are designated 1 and 2, respectively. In each group there are but two mating types; the two in Group 1 are designated I and II, those in Group 2, III and IV. In each of the collections C and D occur some clones of type I and some of type II; only one

of each type is shown in the table. Similarly, in each of the collections W and M occur some clones of type III and some of type IV; but collection P contained only clones of type III, and collections E and U contained only clones of type IV. The clones in the remaining collections (F, G and Q) have not yet been examined sufficiently to be placed with respect to the other collections.

The system of mating types and groups shown in Table 1 resembles that reported for *P. aurelia* by Sonneborn (1937, 1938) in that each group consists of but two interbreeding mating types; and differs from the system reported for *P. bursaria* by Jennings (1938a, 1938b) where four or more interbreeding types are found in each group.

#### CONDITIONS FOR CONJUGATION AND THE MATING REACTION

In both Group 1 (types I and II) and Group 2 (types III and IV) tests were made concerning the relation of the mating reaction to the time of day. In Group 2, mixtures were made at all times of the day and night at hourly intervals and conjugation appeared to occur with equal readiness at all times. In Group 1 hourly tests were made only for the period from 11 P.M. to 6 A.M. Conjugation occurred at all times during this period. However, in mass cultures of this group the characteristic clumping reaction has been observed to occur at all hours of the day. This disagrees with the observations of a number of earlier investigators of *P. caudatum*, who found this species conjugates usually about 4 A.M. Perhaps certain groups show a diurnal periodicity while others do not, as in *P. aurelia* (Sonneborn, 1938) and *P. bursaria* (Jennings, 1939).

The effect of diverse temperatures upon the number of conjugants formed in mixtures of types I and II has been investigated. Since it has been observed that the addition of new culture fluid inhibits conjugation for a time the mixtures of types I and II were placed in four drops of culture fluid before placing at the various temperatures in order that conjugation would not occur before the cultures had come to the temperature being tested. The mixtures



were examined at twelve-hour intervals for conjugants, and all pairs which had formed were counted and removed with a pipette. No conjugation occurred at 9°, 30° or 34° C. The best results were obtained at 18° and 24° C., but it took several days longer to obtain the maximum number of conjugant pairs at 18° than at 24° C. At 27° C. conjugation occurs, but fewer pairs are formed than at 18° or 24° C.

TABLE 2

INTERACTION OF SPLIT PAIRS OF CLONE M, TYPE III, GROUP 2 OF *PARAMECIUM CAUDATUM*. SP3a REPRESENTS MEMBER a OF SPLIT PAIR 3

	WIII	MSP3b	MSP6a	MSP8b	MSP9a	MSP3a	MSP6b	MSP8a	MSP9b	WIV
WIII .....	-	-	-	-	-	+	+	+	+	+
MSP3b .....	-	-	-	-	-	+	+	+	+	+
MSP6a .....	-	-	-	-	-	+	+	+	+	+
MSP8b .....	-	-	-	-	-	+	+	+	+	+
MSP9a .....	-	-	-	-	-	+	+	+	+	+
MSP3a .....	+	+	+	+	+	-	-	-	-	-
MSP6b .....	+	+	+	+	+	-	-	-	-	-
MSP8a .....	+	+	+	+	+	-	-	-	-	-
MSP9b .....	+	+	+	+	+	-	-	-	-	-
WIV .....	+	+	+	+	+	-	-	-	-	-

In Group 2 (types III and IV) the effect of temperature has not yet been investigated, but observations of considerable interest have been made on the effect of diverse nutritive conditions. The various stages of nutritive decline from the well-fed to the starved condition may be represented by the numbers 1 to 5, respectively. When cultures of types III and IV in these diverse nutritive states are mixed, the following behavior is observed:

(1) Animals very well fed and plump. No mating reaction or conjugation.

(2) Animals well fed but not plump. A weak mating reaction; a few pairs cling together but break apart without conjugating.

(3) Animals of moderate size, not well fed. Strong mating reaction: many clumps form; these disintegrate into pairs which fuse and complete conjugation.

(4) Animals small and thin. Strong mating reaction:

many clumps form; these disintegrate, but few or none of the animals proceed to conjugate.

(5) Animals very small and starved. No mating reaction and no conjugation.

#### INHERITANCE OF MATING TYPE

All the clones reproduced true to type for periods of at least two weeks, and all but two for a period of at least one month. Hence the mating types are strictly inherited during vegetative reproduction for considerable periods of time. However, in most of the clones conjugation eventually occurs. In order to ascertain whether such conjugation is the result of the presence in the culture of animals of diverse mating types, pairs not yet firmly united were separated and each of the two individuals separately cultured. Table 2 gives the results of tests for mating types made on four such split pairs removed from a culture of type III of clone M, Group 2. As appears in the table the culture from one member of each split pair conjugated with type III, not with type IV, while the culture from the other member of each split pair conjugated with type IV, not with type III. This shows, therefore, that when conjugation occurs in a clone that has long produced individuals of one type only, it is the result of the production of both mating types within the culture. When individuals of each type are isolated from such a culture, each again reproduces true to type for long periods: a month in the case of the one that was the same type as the original culture, *i.e.*, type III, and at least three months in the case of the new type, IV.

The fact that the type IV cultures from such split pairs remained true to type longer than the type III cultures agrees with observations on the clones isolated from natural collections. Some of the original clones of type IV have continued to reproduce true to type for the entire period of nine or ten months that they have been under observation. This is, however, not true for all clones of type IV, nor is it true for any clone of type III. In Group

1 a somewhat similar condition has been observed in clones isolated from nature. Of five clones of type I, four have remained true to type for a period of nine to ten months. The fifth clone produced a few pairs of conjugants about five months after it was isolated from the wild. Two of the clones of type II which have been isolated from nature give epidemics of conjugation without mixture every two to three weeks.

These genetic relations are similar to those found in *P. aurelia* by Sonneborn (1937; 1938; 1939) and by Kimball (1937): some stocks give rise to both mating types characteristic of their group; others remain permanently of one type. In *P. aurelia* the changes of type within a clone are consequences of nuclear reorganization (endomixis or autogamy). It has also been shown by Giese and Arkoosh (1939) that in one of the mating types of *P. caudatum* studied by them conjugation within the clone occurs only after endomixis; presumably this is the basis of the changes in the present clones of *P. caudatum*, but this matter has not yet been investigated.

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## MATING TYPES IN EUPLOTES<sup>1</sup>

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FROM the previous papers of this symposium, it can be seen that much information has been gained concerning mating and, in particular, mating types and their inheritance in the genus *Paramecium*. It seemed desirable to extend this type of investigation to another ciliate genus not closely related to *Paramecium*. For this purpose, a study was undertaken of the hypotrichous ciliate, *Euplotes*. The work on this organism is still in the early stages, but it is already possible to say that mating types similar to those found in *Paramecium* are found in it also.

The processes occurring during the life of this organism are slightly different from those found in *Paramecium*. The only difference in the process of conjugation that is of any importance for present purposes is that only one macronuclear anlage is formed in each ex-conjugant instead of two or more. Therefore, the terms clone and caryonide in this organism refer to the same unit. No encystment or endomixis has been seen, though careful watch was kept for both. The reports of endomixis-like processes in *Euplotes* that have appeared in the literature to date do not indicate that any replacement of the macronucleus from the micronucleus occurs. The formation of new caryonides at endomixis, therefore, apparently does not occur in *Euplotes*.

To turn to the results of the investigations of mating, when animals of certain clones are mixed, large numbers of conjugant pairs are formed in the mixture. However, in no case, has any immediate clumping together of large

<sup>1</sup> Presented at the joint symposium on "Mating Types and Their Interactions in the Ciliate Infusoria" of the American Society of Zoologists and the Genetics Society of America, in conjunction with the American Association for the Advancement of Science, at Richmond, Virginia, December 30, 1938.

numbers of animals, such as is so characteristic of the mating reaction in *Paramecium*, been observed. In fact, no sticking together of any sort is seen until at least an hour and a half and frequently longer after mixture. After this time, the animals become comparatively quiet and more or less aggregate in the bottom of the depression of the slide in which they were mixed. In appropriate mixtures, pairs of animals then begin to stick together. During this process, there was never observed what could be considered a clear group of more than two animals attached to each other. This process of gradual sticking together of two animals is very different from the sudden sticking together of large numbers of animals that occurs in the mating reaction in *Paramecium*.

The conditions under which conjugation will occur when appropriate clones are mixed are somewhat like those found for certain races of *Paramecium*. The animals mate best when they have been well fed and have almost exhausted the food supply. In practice, it has been found best to add a small amount of food to the mixture when it is made. This food supply is exhausted within a few hours; and, if a suitable mixture was made, the animals start to mate. There was no indication of any effect on mating of the time of day or of temperature within a range of 18° to 31° C. As in some stocks of *Paramecium*, animals that have recently conjugated will not conjugate again until a certain period has elapsed; in *Euplotes*, a period of a month or more.

I will now turn to the results of mixing various clones. In the first place, all the clones studied can be divided into five groups. No mating has been found in mixtures of any clones of different groups. Appropriate mixtures of clones of the same group do, however, contain conjugants. Between some of these groups, there are considerable morphological differences, while between others the differences are slight or non-existent. Though they are all closely related to *Euplotes patella*, no classification of *Euplotes* proposed up to now fits all these groups. I do not at

present wish to go into the taxonomy of *Euplotes* nor to set up species until more material has been studied. I merely wish to point out that the categories that have for present purposes been called groups differ from the groups found in *Paramecium* in being more or less morphologically dissimilar from each other.

Only one of these groups has been analyzed in detail for mating types. In this group, 55 clones have been studied. While all possible mixtures between these clones have not been made, a considerable number have; and from the results of these mixtures it is possible to divide the clones of this group into six mating types. Conjugation occurs in mixtures of clones of different mating type but not in those of the same mating type. As an example, the interactions among 20 clones between which all possible mixtures were made are shown in Table 1. The situation in this group, then, is very much like that found in *Paramecium bursaria*, in which, unlike *Paramecium aurelia*, more than two mating types occur in each group.

The inheritance of the mating types at conjugation has not been fully worked out. However, it is known that, as in *Paramecium*, change of mating types occurs at conjugation. As in *Paramecium bursaria*, clones of mating type diverse from that of either parent are produced as well as those like the parents.

As in *Paramecium*, mating was found in some cases within unmixed samples of one clone. That this mating is not, in all cases at least, due to change of mating type following replacement of the macronucleus at some endomixis-like process is shown by one experiment. Two animals were isolated and each allowed to give rise to a small mass culture. These cultures were examined regularly, and samples were stained every day. No signs of any unusual nuclear changes were observed. Since in *Euplotes* new macronuclear anlagen are clearly visible with low magnifications in the living animal, any replacement of the macronucleus by the formation of a new anlage would have been seen. None was. Nevertheless, conjugation

TABLE 1

THE INTERACTIONS BETWEEN 20 CLONES OF SIX MATING TYPES, ALL BELONGING TO ONE GROUP. THE PLUS SIGN INDICATES THAT CONJUGATION OCCURRED IN THE MIXTURE IN QUESTION; THE MINUS SIGN, THAT IT DID NOT. ALL THE CLONES OF A MATING TYPE ARE GROUPED TOGETHER

	52b	53b	67a	57b	40b	41b	49b	56b	21a	44b	45b	58b	60b	43b	46b	63b	68b	69a	73a	69b
52b	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
53b	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
67a	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57b	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40b	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
41b	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
49b	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
56b	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
21a	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
44b	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
45b	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
58b	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
60b	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+
43b	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
46b	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
63b	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
68b	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
69a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
73a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
69b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-

occurred within these two cultures. It must be concluded that the vegetative progeny of a single individual can mate with each other, in some cases, though no replacement of the macronucleus has taken place. Whether this involves a change of mating type during vegetative reproduction such as has been found in *Paramecium aurelia* has not yet been determined.

In Euplates, it has been possible to show that in many mixtures in which mating occurs this mating is not only between animals of different clones but also between those of the same clone. This unexpected relation was demonstrated by the use of double animals. In order to make the

following discussion clear, a brief description of these animals will be given.

In protozoan literature, there have been reports from time to time of the occurrence of abnormal animals composed of two individuals fused together in some manner. Some of these double animals have been so constructed that when they divided two double animals like the parent were produced. Such "stable" double animals can, then, give rise to a clone all the animals of which are double. The double *Euplotes* that was used in this work was of this sort. It is composed of two animals fused by their right aboral surfaces and is homopolar. The two fused animals instead of remaining more or less flat like normal single animals are bent through a considerable angle along the line of fusion.

The oral surfaces of the two halves of a double are free; and, therefore, it is physically possible for the doubles to conjugate with other animals. Actually in appropriate mixtures with single animals, conjugation between the single and the double animals does take place. However, in these same mixtures very frequently conjugant pairs composed of two single and more rarely of two double animals occur. This means, then, that animals of the same clone are mating with each other. Nevertheless, unmixed samples of these clones, set aside at the same time that the mixtures were made, contained no conjugants. Thus mixture of one clone with another appears to induce conjugation between animals that otherwise would not have conjugated with each other.

Further light on the way in which this induction was brought about was obtained by mixing animals from one clone with culture fluid (without animals) taken from a culture of another clone. This culture fluid was pipetted off from the culture and examined carefully for animals. All that were found were removed. Animals of the clone to be tested were then added to it. In some cases, considerable conjugation occurred among these animals, though none was found among animals from the same clone un-



mixed with foreign culture fluid. Culture fluid from clones of single as well as double animals had this effect. It must be concluded that certain clones, perhaps all, have the ability to so change the fluid in which they are growing that it will induce conjugation among animals of certain other clones.

Sonneborn has found in *Paramecium aurelia* that culture fluid from one caryonide does not induce conjugation among animals of another. However, he finds that contact with an animal of opposite type makes an individual capable of forming temporary pairs with animals of its own type. These pairs do not last long, however, so that final conjugation occurs only between animals of opposite type. In *Euplotes*, on the other hand, pairs formed between animals of the same clone regularly complete conjugation.

What the relations are between the induced mating within a single clone of *Euplotes* and the mating types is not certain. It is clear, of course, that two clones of the same mating type do not induce mating within each other since when they are mixed no conjugation occurs. In many mixtures of clones of double and single animals, conjugation takes place between the doubles and the singles; and in a few mixtures this is the only sort of mating that has been seen. Therefore, not all mating within mixtures is mating within a clone induced by the presence of animals or fluid from another clone. However, it is too early to say whether there are any other systematic relations between induced mating and the mating types.

# THE VISIBLE ORGANIZATION OF THE GIANT SALIVARY GLAND CHRO- MOSOMES OF DIPTERA

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It is well known that ordinary chromosomes of animals and plants, when in the condensed mitotic stages, contain coiled chromonemata, often clearly visible under the microscope. Such chromonemata were identified as early as 1908-1911 by Bonnevie (1908) and by Vejdovsky, and were familiar to Alverdes (1912) when, in his studies on dipteran salivary gland nuclei, he described the organization of the giant "spireme," which is now known to represent giant chromosomes. Alverdes naturally attempted to interpret the structure of these giant bodies in terms of the chromonemata found in "ordinary" chromosomes. But in doing this he was led to a conception which has subsequently had to be abandoned. Since the view was quite in keeping with conceptions then current, however, it was readily accepted and was supported for a long time by other observers, thus serving to delay recognition of the true significance of these structures for twenty years. .

More recently other observers, although not accepting the particular view of Alverdes, have likewise attempted to interpret the organization of these giant chromosomes in terms of *visible* chromonemata like those seen in "ordinary" chromosomes. And from their accounts it is difficult to avoid the conclusion that these authors have similarly been led to their views largely through the influence of what is seen in "ordinary" chromosomes. Such seems to be the case not only as regards the interpretation of Koltzoff (1934) and of Bridges (1935), who described "chromonemata" coiled around a central axis in these chromosomes (a view now abandoned) (Bridges, *a*) but also as regards that of Koller (1935), Bauer (1936) and Painter and Griffen (1937), who described "chromone-

mata" here in larger numbers, extending diagonally or straight along the chromosome, and filling it uniformly. Although it is, of course, important to determine the relationship between the constituents of the giant chromosomes and those of other chromosomes, it is not necessarily true that these constituents will show the same morphological characteristics in the two cases. Indeed, there is strong evidence that they do not.

As indicated in earlier papers (Metz, 1935, 1937; Metz and Lawrence, 1937), our evidence in this laboratory indicates that the giant chromosomes present a distinctly different problem from "ordinary" chromosomes, as regards structure, and that the pattern discernible in the former is not made up of visible chromonemata. The problem has been treated in the papers just referred to (Metz, 1935, 1937; Metz and Lawrence, 1937); but since there still seems to be some confusion as to the nature of the evidence and of the conflicting interpretations, a brief discussion of certain points is presented here. The discussion concerns especially (1) comparison of the giant chromosomes with "ordinary" chromosomes, and (2) comparison of current views as to the structure of the former. Since Koller (personal communication) has abandoned his earlier view and adopted that of Metz and Lawrence, the latter comparison involves three interpretations, that of Bauer, that of Painter and Griffen and that of Metz and Lawrence.

Since different investigators of salivary gland chromosomes have used different genera of flies, the writer has made a careful study of conditions in the four genera used (*Chironomus*, *Drosophila*, *Sciara*, *Simulium*) to make sure that, as is actually found to be the case, the structure is fundamentally the same in all.

A salivary gland chromosome represents a pair of homologous chromosomes fused side by side to form an essentially cylindrical body which, in the larger nuclei, is approximately a hundred times as long as, and thousands of times the volume of, the comparable chromosomes in "ordinary" somatic cells at metaphase. The chromosomes are coiled about in the nucleus, and, unlike those of ordi-

nary cells, they occupy practically the entire volume of the nucleus (Doyle and Metz, 1935; Buck and Boche, 1938). The latter fact is especially significant in the present connection, for, together with other evidence, it shows that most of the nucleoplasm is here contained *within* the chromosomes, making the morphological problem presented by these chromosomes distinctly different from that presented by "ordinary" chromosomes in cells capable of mitosis.

The primary pattern, visible under the microscope in the salivary gland chromosome, is made up of transverse chromatic disks or "cross-bands," and in a gross sense is constant for each chromosome, the disks presumably reflecting in some manner the linear seriation and localization of the genes. Present interest centers partly in the nature of these disks, but more especially in that of the faint longitudinal lines which extend from one disk to the next across the intervening achromatic zones (*D* and *E*, in Fig. 1).

The salivary gland chromosome is commonly considered as a multiple structure, owing its size to repeated divisions of the chromonemata present before the enlargement began. There are good theoretical grounds for this view, although no demonstrative evidence has yet been secured. On the basis of size, either of chromosomes or of nuclei, however, the number of chromonemata here should be a thousand or more, rather than the small numbers described on the basis of visible constituents. According to Bauer the faint longitudinal lines just referred to are true threads and represent true chromonemata. According to the revised view of Painter and Griffen (1938) they are true threads, but represent bundles of chromonemata in which the individual chromonemata are sub-microscopic and hence invisible. On these two views the granules or chromatic droplets represent either individual chromomeres (Bauer) or bundles of sub-microscopic sister chromomeres (Painter and Griffen), aligned on the chromonemata.

The transverse chromatic disks, often granular in appearance, together with the delicate longitudinal lines,

make up a visible pattern which varies under different conditions according to the distribution of the chromatic material and, presumably, degree of internal pressure. Sometimes the disks appear as relatively sharp, straight cross-bands, and the longitudinal lines are faint (Fig. 2, A). In other cases the disks appear as zigzag bands and the longitudinal lines are prominent, as represented sche-

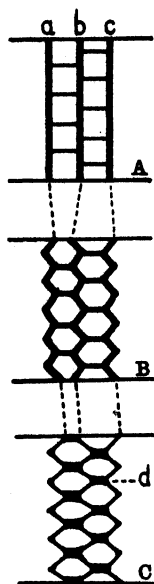


FIG. 2. Diagram illustrating how the appearance of one region may vary with differences in the distribution of chromatin and, presumably, differences in internal pressure. In A the disks (a, b, c) are essentially straight and contain most of the chromatin. (Actually, of course, they are not as smooth in outline as shown here.) In B the disks are zigzag in outline and the achromatic droplets hexagonal in optical section (as in Fig. 1 and in Fig. 3 at c). In C the chromatin, instead of being mainly in the disks, lies mainly in the spaces between the achromatic droplets separating the disks. Here it forms conspicuous granules, each of which contains material from two disks (compare region d in photograph in Fig. 3). These conditions are not hypothetical, but are actually observed.

matically in Fig. 1 and Fig. 2, B. Between these two extremes is a complete range of intermediate conditions.

According to the interpretation of Metz and Lawrence (1937) the giant chromosome, at least in the fixed condition, is made up of a series of chromatic disks which,

although connected by true chromosome material, are separated by layers of achromatic droplets presumably representing the nucleoplasm or "matrix" material. Each two successive disks are separated by one layer of droplets embedded in the true chromosome substance, which extends longitudinally between and around the droplets from the one disk to the next, as indicated in Fig. 1. Ordinarily, of course, only a few adjacent droplets are seen in median optical section at one focal level, as seen at *c* in Fig. 3, instead of a large number as shown in the diagram. Likewise, the number of droplets, like that of the granules, actually differs widely at different loci along the chromosome instead of being uniform as shown here. Otherwise, however, the diagram is essentially accurate.

On this view the delicate longitudinal lines (*D*, *E*, Fig. 1) are not threads, as considered by Bauer, Painter and Griffen and earlier authors, but are optical sections of the material lying between the droplets. This material may, of course, be made up of a multitude of sub-microscopic chromonemata tightly packed together; but the visible image is one of continuous substance, and the total pattern is that of an alveolar organization, modified by the presence of the disks.

This interpretation has two advantages: First, it conforms to what is actually observed under the microscope; second, it serves to explain the widely different interpretations given by the other authors noted above. No attempt will be made here to elaborate details as to the nature of the disks, granules, etc., but a few points may be noted. The disks evidently represent regions of high concentration of nucleic acid, and are made of material which is much more resistant to distortion than that of the intervening zones. Presumably the achromatic material is produced within the chromosome during its development. Apparently there are at least two fundamentally different types of chromatic granules. In one type each granule contains material from two different disks (Metz, 1935, p. 491; Metz and Lawrence, 1937) (Fig. 2, C).

Not enough emphasis has been laid on the fact that no criterion is known for distinguishing a really *single* disk or telling how many disks are actually involved in many if not most of what are ordinarily referred to as individual disks. Many of the latter are known to be compound, but the extent of the complexity is unknown. Until such fea-

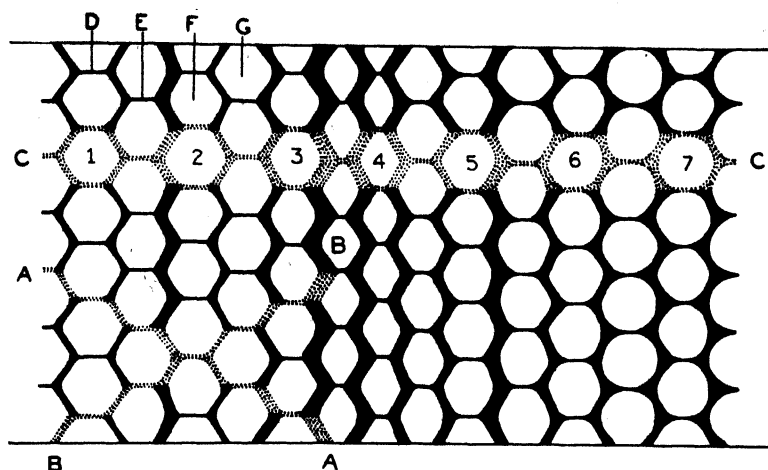
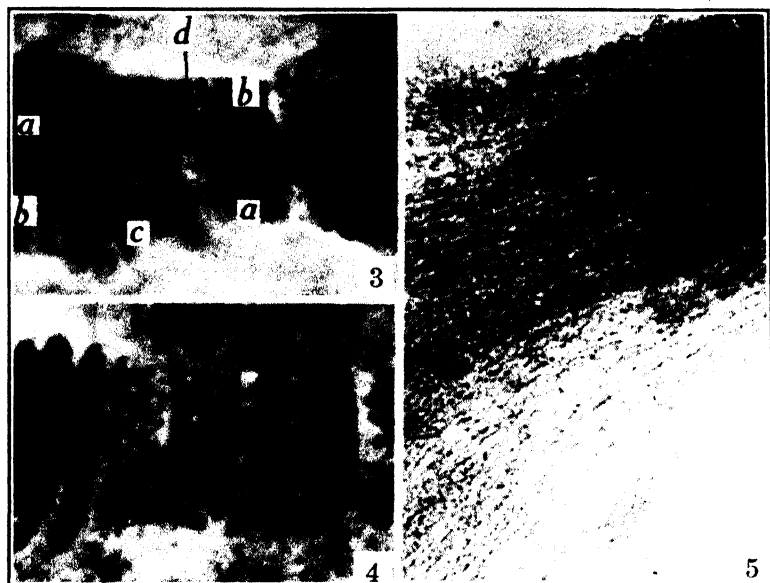


FIG. 1. Diagram of internal structure of salivary gland chromosome according to the interpretation of Metz and Lawrence. Compare with the photographs shown in Figs. 3 and 4. The diagram indicates also how this interpretation serves to explain the conflicting views of other authors. The transverse (vertical) zigzag bands represent the chromatic disks, separated from one another by achromatic droplets, which, in optical section, may appear spherical, as at the right, or hexagonal, as at the left. Compare the latter region with region c in the photograph in Fig. 3. The diagonal zigzag lines, such as A-A and B-B, represent what some authors (*e.g.*, Koltzoff, Bridges, Bauer) have considered to be chromonemata. The series of lines and spaces C-C illustrate what Painter and Griffen consider to be a chromonema (bundle).

tures are cleared up the real nature of the "granules," etc., will remain obscure. A disk may be made up of a multitude of sub-microscopic chromomeres, but according to our evidence no chromomeres of genetic or biological significance are visible. Rather, the granules, etc., appear to be due to localized distortions of the disks.

Such an interpretation not only conforms to the evidence, but also seems entirely in keeping with expectation when the size and general nature of the giant chromosomes

are considered. If a chromonema in an "ordinary" dip-  
teran chromosome were extended to the length of a sali-  
vary gland chromosome it should be sub-microscopic  
throughout its length.



FIGS. 3, 4, 5. Photographs of chromosomes and cytoplasm from aceto-  
carmine smear preparations of salivary glands of *Chironomus* sp. Figs. 3  
and 4,  $\times 2500$ ; Fig. 5,  $\times 1134$ . Fig. 3. Portion of one chromosome (pair)  
showing details of structure. Note the zigzag lines extending diagonally  
between *a* and *a* and between *b* and *b*, forming a crisscross pattern (compare  
with Fig. 1, A-A, B-B). Also note the relation of these lines to the honey-  
comb type of pattern shown at *c*, where enough of the achromatic droplets  
happen to lie in one plane to reveal the pattern clearly (compare with Figs.  
1 and 2, B). At *d* rows of granules are present like those shown schemati-  
cally in Fig. 2, C. Fig. 4. Portion of another chromosome (pair) in which  
the diagonal crisscross lines are accentuated by slight distortion. The criss-  
cross pattern is present at all optical levels as one focusses up and down,  
showing that the lines can not represent chromonemata. Fig. 5. Stretched  
cytoplasm, showing how distortion produces granular thread-like striations  
like those produced by similar means in the chromosomes and thought by  
some authors to be chromonemata.

In comparing the view of Bauer with that of Painter and  
Griffen, it is to be noted first that the actual part of the visi-  
ble pattern which Bauer calls a chromonema is distinctly



different from that which the latter authors call a chromonema (bundle). This has been determined not only by study of our material, but by examination of preparations kindly provided by Dr. Bauer and Dr. Painter. The essential aspects of the difference are shown schematically in Fig. 1, where the diagonal lines, such as *A-A* and *B-B*, represent the "chromonemata" of Bauer, and the longitudinal series of lines and spaces, such as illustrated by *C-C*, represent the "chromonemata" (bundles) of Painter and Griffen.

Although the lines of Bauer often do seem thread-like in appearance after certain fixatives, it seems clear from several lines of evidence that they can not be chromonemata, and, indeed, are not actually threads. *E.g.*, (1) Viewing the chromosome from the side and focussing up and down, these lines are seen to form a diagonal criss-cross pattern at all optical levels, indicating that they are a part of a geometrical, three-dimensional system—a meshwork or spongework reflecting an alveolar structure, as indicated in Figs. 1, 3 and 4. (2) Individual lines extend across both members of the pair of chromosomes, whereas a chromonema is part of a single chromosome. (3) The number of lines, like that of the granules, differs greatly in different parts of a chromosome and is not what would be expected if they represented chromonemata, as indicated in earlier papers (*l.c.*). Other reasons are noted below in connection with the interpretation of Painter and Griffen.

Since Painter and Griffen now agree with Metz and Lawrence that no individual chromonemata are detectable in the giant chromosomes the difference in interpretation here is not very significant. In attempting to throw light on the physical nature of genes and chromonemata it makes relatively little difference whether we consider a disk as made up of a thousand or more individually invisible units (chromomeres) or of a few granules or "chromomeres," each of which is itself made up of a large number of these invisible units. It should be noted, however, that our evidence does not support the view of Painter and Griffen.

This feature will be treated more fully elsewhere; but a few points may be noted briefly here, as follows: (1) If individual bundles of chromonemata were formed, as postulated by Painter and Griffen, the number should be uniform throughout the chromosome. But, as is well known, the number of longitudinal lines, of chromatic granules and of heavy-walled droplets or so-called "chromomeres" differs widely in different parts of a chromosome. This is true during the development of the chromosome as well as at the end. Also, we have been unable to find evidence that the numbers are regularly multiples of two (8, 16, 32, etc.) as postulated. (2) The evidence of Painter and Griffen is based primarily on severely stretched chromosomes. According to our observations, such evidence is unreliable. In such material the more delicate structure is distorted and gives the appearance of strands, connecting the heavier granules and heavy-walled droplets in the more chromatic regions. The same type of image is produced by stretching the cytoplasm of these same cells (Fig. 5). Moreover, if these delicate lines were really chromonemata they should be thicker and very conspicuous in less stretched conditions, which is not the case. (3) A bundle of chromonemata, on the view of Painter and Griffen, is said to be represented by a continuous thread, on which are aligned granules and conspicuous heavy-walled droplets called "chromomeres." Thus in the pattern of the relaxed chromosome, as shown in Fig. 1, spaces 1 to 7 represent the "chromomeres" of one "chromonema" bundle, and the corresponding spaces at levels 1, 2, 3, etc., represent the "chromomeres" of the other "chromonemata" bundles. Hence, line *D* and the other lines in this transverse (vertical) row would represent the sides of "chromomeres," while *E* and its counterparts would represent threads. Similarly, space *F*, like 2 and its other counterparts, would represent a "chromomere," whereas *G*, which looks just like it, would merely represent a space between two transverse rows of chromomeres. We are unable to find justification for these distinctions (see Fig. 3). (4) By stretching the chromosome diagonally, side-

wise, etc., the components of the pattern within the chromosome may be lined up into so-called "chromonemata" extending in any desired direction. (5) At some loci conspicuous granules lie *between* what are said to represent chromonemata. Such a condition is shown in Fig. 2, C, which may be interpreted for this purpose by comparison with Fig. 1. *E.g.*, position *D* in Fig. 1, on the view of Painter and Griffen, lies between abutting "chromomeres" of two adjacent chromonemata. This is the position occupied by the granules, such as *d*, in Fig. 2, C. In some cases the droplets, between which such granules lie, are heavy-walled and are clearly the type of structures called "chromomeres" by those authors. (6) According to our evidence from direct observation and also from study of small deficiencies the so-called "chromomeres" are not biological units. It takes two disks to make a transverse plate of "chromomeres," as indicated in Fig. 1.

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## SHORTER ARTICLES AND DISCUSSION

### THE GENETICS OF NON-EPITHELIAL TUMOR FORMATION IN MICE

THE present communication represents a condensed summary of a series of experiments on the genetics of the tendency to produce non-epithelial tumors in mice.

A total of 343 such tumors occurring spontaneously in two inbred strains of mice and their hybrids is included in the recorded data. Each tumor has been examined and identified histologically.

In one of the parent inbred strains C57 black there were 121 such tumors. In the other dba, dilute brown there were 23.

In the C57 black stock the tumors may be tabulated as in Table 1.

TABLE 1

Type of animal	Total mice	Non-tumor	Non-epithelial tumor	Per cent. showing non-epithelial tumor	Mean age at death non-tumor	Mean age at death tumor	Per cent. of non-epithelial tumor in lymphatic system	Per cent. of non-epithelial tumors in liver
Breeding ♀ . . . . .	570	499	64	11.22	608.1	706.3	57.8	25.0
Virgin ♀ . . . . .	133	109	26	19.54	813.7	711.0	65.4	19.2
Males . . . . .	174	142	31	17.81	720.2	741.5	67.7	32.3
<b>Total . . . . .</b>	<b>877</b>	<b>750</b>	<b>121</b>	<b>13.79</b>				

The first point to be noted is that there is no significant difference in the incidence of tumors between breeding females, virgin females and males. This is a marked contrast with the behavior of epithelial mammary tumors, which are confined to the female sex and which are much commoner as a general thing in breeding females than in virgins.

The twenty-three tumors which occurred in the other parent stock (dba) were located as follows: lymphatic, 82.6 per cent.; liver, 4.3 per cent.; other regions, 13.1 per cent.

#### HYBRIDS BETWEEN C57 BLACK AND DILUTE BROWN STOCKS

The only types of hybrids included in this report are virgin females. There are four groups of such animals.

These groups are as follows: F<sub>1</sub> produced from a cross of dilute

brown females by C57 black males ( $dBF_1$ );  $F_1$  from the reciprocal cross of C57 black females by dilute brown males ( $BdF_1$ ),  $F_2$  hybrids produced by inbreeding  $dBF_1$  mice ( $dBF_2$ );  $F_2$  animals derived from matings of  $BdF_1$  animals ( $BdF_2$ ).

The numbers recorded for each of the four types are given in Table 2.

TABLE 2

Hybrids	Non-tumor	Tumor	Total
$dBF_1$ .....	37	5	42
$BdF_1$ .....	158	43	201
$dBF_2$ .....	407	61	468
$BdF_2$ .....	571	90	661
Total hybrids ....	1,173	199	1,372

The location of the tumors in the four hybrid generations can be shown in Table 3.

TABLE 3

	Lymphatic system	Liver	Other sites
$dBF_1$ .....	40.0	0.0	60.0
$BdF_1$ .....	37.2	4.7	58.1
$dBF_2$ .....	44.3	1.6	54.1
$BdF_2$ .....	36.7	13.3	50.0

The distribution in the hybrids is distinctly different from that in the two parent strains. These are given in Table 4.

TABLE 4

	Lymphatic	Liver	Other sites
C57 Blk .....	62.0	25.6	12.4
Dilute brown .....	82.6	4.3	13.1

From these figures it is clear that the hybrid animals show a much more scattered distribution of tumors than do the pure strains. In this respect there is no outstanding difference between the reciprocal crosses. Both show between 49 and 61 per cent. of the tumors in situations other than the lymphatics or liver, while the pure strains showed between 12.3 and 13.2 per cent.

As regards the *types* of tumors there are also some interesting data.

The pure dilute brown stock included in its total of 23 tumors only two types. The C57 black stock in 121 tumors included nine types. The five  $dBF_1$  tumors were of two types, while the  $dBF_2$

generation had 8 types. The B $\delta$ F $_1$  generation with forty-three tumors showed eleven types, and the B $\delta$ F $_2$  had eleven types.

If one tabulates these data according to the derivation of the maternal line of descent he obtains the following:

Types			
Dilute brown	2	C57 black	9
dBF $_1$	2	B $\delta$ F $_1$	11
dBF $_2$	8	B $\delta$ F $_2$	11

The numbers are not sufficiently large to provide conclusive results, but the suggestion is that the types of tissue affected seem to depend somewhat more upon the maternal derivation of the hybrid than upon the paternal. It would be interesting, therefore, to carry on further experiments to see whether in this respect the difference was a real one and whether it was influenced by foster nursing, as Bittner has found the incidence of mammary epithelial tumors in mice to be.

As regards the possible relationship between coat color and the incidence of non-epithelial tumors, there is only an indication of a situation that may contain interesting possibilities. This is the incidence of such tumors in intense and dilute mice. It can be tabulated as in Table 5.

TABLE 5

	Intense			Dilute		
	Non-tumors	Tumors	Per cent. tumor	Non-tumors	Tumors	Per cent. tumor
B $\delta$ F $_2$ & dBF $_2$ ..	732	105	11.4	236	46	16.3
Little 1934 ...	264	13	4.7	115	10	8.0
Total .....	<b>996</b>	<b>118</b>	<b>10.6</b>	<b>351</b>	<b>56</b>	<b>13.8</b>

If there is a real excess of tumors among dilute mice it is probably due to some physiological relationship rather than to genetic linkage. Its significance is, of course, very doubtful and must await larger numbers for confirmation.

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NOTE OF MODIFICATIONS IN THE MORPHOGENESIS  
OF *DROSOPHILA MELANOGASTER* OCCURRING  
UNDER NEUTRON BOMBARDMENT<sup>1</sup>

For several years the authors have been engaged in a fairly extensive qualitative and quantitative investigation of the incidence of white mosaic patches in the compound eyes of imagoes of *Drosophila melanogaster* exposed to x-rays as eggs or young larvae (Enzmann and Haskins, 1935, 1936). The study was undertaken originally as a statistical one, designed to apply the "sensitive volume" technique to a determination of the magnitude of the genic locus involved in the production of white eye color. It soon became apparent, however, that many results of interest could be derived outside of the main line of research from the same types of experiment, notably in connection with the morphogenesis of the adult organs in the larvae of *Drosophila melanogaster*. In consequence, the original research has been extended to include descriptive studies of the qualitative nature of modifications produced by x-rays in developing larvae of the fruit-fly, such modifications becoming evident, for the most part, in the resulting imagoes. Results of such studies, involving a number of strains of fruit-fly, have been and will be published elsewhere (Enzmann and Haskins, 1937).

It became of considerable interest, in this connection, to conduct parallel, and similarly descriptive and qualitative, studies of imagoes of *Drosophila* which had been subjected to neutron bombardment at the same stage of the life cycle. Thanks to the courtesy of Dr. Ernest O. Lawrence it has been possible to carry out such work at the Radiation Laboratory of the University of California, and the generosity of Mr. Everett R. Dempster in collecting eggs, in hatching them and in mailing the developing larvae to us, and of Mr. Paul C. Abersoeld in exposing them to fairly high dosages of neutrons, has permitted the gathering of considerable data. These have shown such marked, and even striking, deviations, qualitatively, from the similar results found with x-rays as to seem worthy of a note at the present time.

The procedure involved in gathering eggs, in segregating retained ova from those freshly fertilized, in rearing the larvae and examining the adults, was essentially that which has been described elsewhere (Enzmann and Haskins, 1935, 1936), in connec-

<sup>1</sup> From the Haskins Laboratory, Schenectady, N. Y., and the Biological Laboratories, Harvard University, Cambridge, Massachusetts.

tion with the x-ray studies. We benefited greatly in this connection from technique in egg collecting devised by Mr. Dempster.

The types of effects observed in the resulting imagoes were quite different in kind, for the most part, from those observed in x-rayed stocks. Reduplication of organs has been found in a very high percentage of modified flies, an effect observed rarely in x-rayed stocks, and suggestive of a modification of possible "organizers" by neutron bombardment. These duplications have been found to involve characteristic bristles, arista, antennae, compound eyes, eye rims, legs, wings, halteres and abdominal tergites, and very probably included certain internal organs, which have not, however, as yet been investigated.

It has been possible to collect a rather completely graded series of duplications of the compound eye and of the eye rim, the incidence of such modifications being surprisingly high. These range from a slight constriction of a single eye occurring along a very definite line and possibly corresponding to the boundary line between the upper and lower lobes of the normal eye, to complete separation, either into two eyes with a single rim or into two completely independent structures. Several cases have been observed in which a complete and perfect accessory eye was formed, mounted on a stalk projecting from above the labrum, the lateral compound eye being normal. Histological study indicated that the accessory, centrally placed organ was connected to the brain by a distinct optic tract, and was presumably functional. It was not a heritable variation, of course, occurring purely in somatic tissue.

The rim of the eye is likewise subject to reduplication, in a fashion apparently relatively independent of that of the eye itself. Usually the partial or complete splitting of the eye is accompanied by separation of the rim. When the eye is fully divided, both halves may be included in the same rim. When the accessory eye has reached a certain and apparently fairly definitely critical size, a separate rim is acquired. The rim, however, may be divided when the eye is not. Two, three or even four eye rims have been found in a single fly, some bearing a few facets, and others entirely empty. Such results are suggestive of the lack of dependence of the development of the eye rim upon that of the eye proper, and its possible origin from a separate anlage, localized in the peripodal membrane of the eye disc.

Antennal duplications are of common occurrence and consider-



able interest. Usually a complete and normal antenna is found in its proper location, and an accessory structure occurs, situated either very close to, or actually within, the compound eye. The accessory structure, in such cases, ordinarily occupies a very definite position, usually in a notch of the compound eye directed toward the base of the normal antenna and devoid of facets. The accessory antenna has a tendency to normal development when situated outside of the compound eye, but is often represented by a mass of tumor-like cells, revealing their origin as antennal tissue by pigmentation and the character of the hair structures, when occurring within the area of the eye. This too is suggestive of "organizer" influence.

Interesting cases of partial duplication of organs have been found. Halteres are often found "twinning" and mounted upon a single basal segment. Modification of the haltere to wing tissue has been found. Reduplication of legs and true wings has likewise been observed. Secondary fusion of duplicated parts sometimes occurs. No attempt at a theoretical interpretation of the detailed nature of any of these changes has been attempted at this stage of the investigation.

It seems indicated that the studies with neutron beams may constitute an extremely useful tool in morphogenetic investigations of this character, and it is hoped to carry the work much further.

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#### THE FACET FREQUENCY DISTRIBUTION IN BAR-EYED DROSOPHILA

THE extensive studies on facet number in the Bar series of *Drosophila* involving, as they do, statistical methods in the reduction of data make it desirable that some knowledge of the form of the facet frequency distribution be obtained. With the exception of a graphic presentation of data on Bar in an early study by Zeleny (1920) in which a marked positive skewness is disclosed, no statistical analysis has been given.

The present paper deals with an analysis of the facet frequency distributions in two highly inbred but unrelated Bar-eyed stocks which have been used in a number of experimental studies by the writer. The data for the facet distributions were taken from the control flies in a series of experiments carried out at 28° C. As far as possible all environmental factors known to effect facet number, *e.g.*, temperature, food, crowding, were carefully controlled. The residual variability in both stocks may be considered largely as the resultant of unknown factors acting at random on the facet-forming processes, coupled with slight fluctuations in the known environmental factors cited above.

The analysis of the data involved the calculation of the first four moments of the distributions and the derivation from these of the statistical constants with their standard errors given in Table 1. The analytical constants  $\sqrt{\beta_1}$  and  $\beta_2$  and the criterion,

TABLE 1  
ANALYTICAL CONSTANTS FOR FACET FREQUENCY DISTRIBUTIONS

	A males		A females		B males		B females	
N	426		354		531		510	
Mean $\pm$ s.e.	50.81	$\pm 0.329$	48.50	$\pm 0.357$	68.81	$\pm 0.335$	52.45	$\pm 0.224$
$\sigma \pm$ s.e.	6.79	$\pm 0.233$	6.71	$\pm 0.255$	7.72	$\pm 0.237$	5.07	$\pm 0.159$
$\sqrt{\beta_1} \pm$ s.e.	0.3624 $\pm 0.1187$		0.3032 $\pm 0.1302$		0.1517 $\pm 0.1063$		0.1845 $\pm 0.1085$	
$\beta_2 \pm$ s.e.	2.8760 $\pm 0.2374$		3.0430 $\pm 0.2604$		2.8283 $\pm 0.2126$		2.9010 $\pm 0.2170$	
$\kappa_1 \pm$ s.e.	-0.6419 $\pm 0.4758$		0.1897 $\pm 0.5208$		-0.4131 $\pm 0.4157$		-0.3001 $\pm 0.4339$	
$\chi^2$	22.8088		26.6785		18.0697		15.7319	
Degrees of freedom	15		16		14		13	
$\rho$	0.0889		0.0455		0.2039		0.2649	

$\kappa_1$ , serve to establish the curve type which may best be used to fit the data. For the normal distribution  $\sqrt{\beta_1} = 0$ ;  $\beta_2 = 3$ ; and  $\kappa_1 = 0$ . The values of  $\sqrt{\beta_1}$ , which serves as a measure of skewness, are small and positive but deviate significantly from zero in stock A. In stock B the deviations from zero are statistically insignificant. One may conclude, therefore, that the facet distribution in stock A shows a slight positive skewness.

$\beta_2$ , which is a measure of kurtosis or peakedness in the distributions, does not deviate significantly from 3 in either stock; nor does  $\kappa_1$ , which is a function of both  $\beta_1$  and  $\beta_2$ , deviate significantly from 0. Despite the slight positive skewness in stock A, it is clear that the normal curve of frequency may be applied to the data as a satisfactory approximation.

The calculated curves and observed points for each of the four sets of data are presented in Fig. 1. Goodness of fit was tested

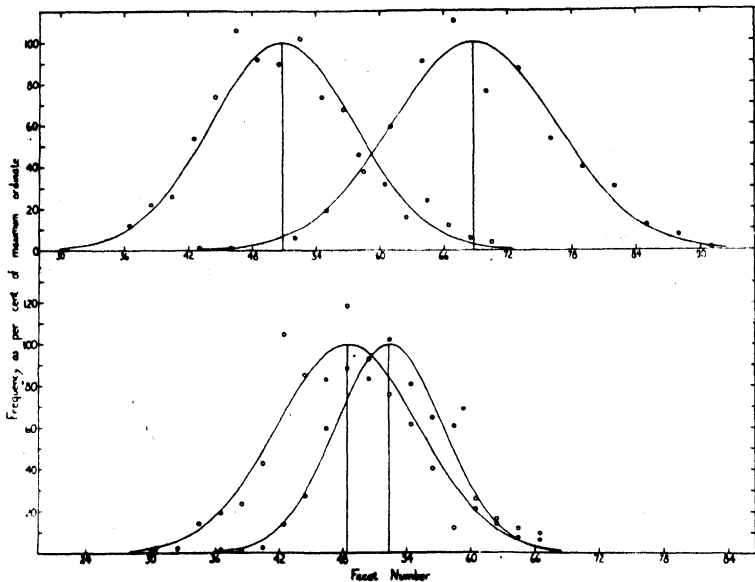


FIG. 1. Normal frequency curves fitted to facet data of males and females of Stocks A and B. *Upper Curves*—Males; *Lower Curves*—Females. *Open Circles*—Stock A; *Shaded Circles*—Stock B.

by the  $\chi^2$  method. The values of  $\chi^2$  and the corresponding probabilities are given in Table 1. The probabilities were taken from Elderton's (1901-02) tables using  $(\eta' - 2)$  degrees of freedom. It is apparent from the probability values that the data for stock B give a much better fit to the normal curves than the data for stock A. This is to be expected, in view of the slight positive skewness in the data from stock A. Interpreting the probability values in the conventional manner we may say for stock A males that about 10 of 11 random samples should give as good a fit or better, while for A females about 21 of 22 random samples should give as good a fit or better. For stock B males 4 out of 5 random samples should give as good a fit or better; for the females about 3 out of 4 random samples should give as good a fit or better.

The better fit of data on stock B to the normal curve may be due to two factors, (1) the greater genetic homogeneity of the stock as shown by a lower parent-offspring correlation (Margolis,

1936) and (2) better control of environmental factors, particularly crowding, which tends to reduce facet number. Since experiments with stock B were undertaken several years after those with stock A, improvements in experimental technique have probably played an important part.

Zeleny, in the study previously cited, attributed the skewness observed in his distributions to the fact that factors which modify facet number operate exponentially, that is, a ten-facet change in a fly having one hundred facets is equivalent to a twenty-facet change in a fly having two hundred facets. By using a logarithmic scale in setting up class intervals (each class interval taken as a fixed per cent. of the midvalue of the class) Zeleny found that a very satisfactory approximation to a symmetrical distribution resulted. This led to the development of a factorial system of notation for facet numbers in which factorial units represented logarithmically equal facet classes. These factorial units were accepted as a better measure of the factors affecting facet number than facet number itself.

It is clear that the data presented here are symmetrically distributed without recourse to the logarithmic seriation of data recommended by Zeleny. This difference in the two sets of data requires brief comment. In the earlier work crowding had not yet been recognized as a factor which affects facet number. Since this factor causes a reduction in facet number and does not operate at random on the population of developing flies it is precisely the type of environmental factor which will lead to positive skewness. Those flies affected by crowding are shifted progressively toward the low end of the facet distribution. From this point of view, recourse to hypothesis concerning the quantitative effect of any factor which modifies facet number is unnecessary. It is doubtful, moreover, whether a predictable relation can be established between the form of the distribution of a character in a population and the nature of the effect of modifying factors which affect that character, provided that these factors operate at random on members of the population. The factor of crowding, however, operates selectively on a certain proportion of the population and inasmuch as it causes reduction in facet number leads to positive skewness in the facet distribution.

The analysis presented here demonstrates that the normal frequency curve gives an adequate description of the facet distribution in genetically homogeneous Bar-eyed stocks raised under

satisfactory environmental conditions. Data dealing with the effect of crowding on the form of the facet frequency distribution will be analyzed elsewhere.

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### AN EVOLUTIONARY CHANGE IN CHROMOSOME SHAPE IN SCIARA

#### INTRODUCTION

IF we leave out of account the peculiar "limited" chromosomes of *Sciara*, which are not involved in the present discussion, there are two common types of chromosome group in this genus. Both have eight chromosomes. One consists of two pairs of V's and two pairs of rods; the other, of one pair of V's and three pairs of rods. Each type is found in several species (Metz, 1926, and unpublished data). Here, as in *Drosophila*, therefore, it seems evident that rod-like chromosomes become transformed into V's, or V's into rods. The present account deals briefly with the question as to how this transformation is brought about. It is concerned especially with conditions in *S. ocellaris* Comstock. Comparisons of four different "wild" strains have shown that both types of chromosome group are found in this species. The strains are all interfertile. In the present account the results of different crosses are reviewed, together with evidence from study of the salivary gland chromosomes. A description is also given of the chromosome group of the closely related species, *S. reynoldsi* Metz.

Of the various species of *Sciara* studied to date in this laboratory, *S. ocellaris* and *S. reynoldsi* are the only two which do not possess the so-called "limited" chromosomes (see Metz, 1926, 1938b) and also the only two which are partly interfertile. The cross, *S. reynoldsi* female by *S. ocellaris* male, has never yielded offspring; the reciprocal cross, however, does give an F<sub>1</sub> generation, including males, females and gynandromorphs. (Metz and

Lawrence, 1938, and unpublished data.) Further indication of the close relationship existing between these two species is found in the striking similarity in their external morphology (Metz, 1938a) and also in the fact that the sex-linked character "yellow" in *S. reynoldsi* is allelic to "yellow" in *S. ocellaris* (Crouse and Smith-Stocking, 1938).

The author is indebted to Dr. C. W. Metz for the encouragement and many helpful suggestions he has given throughout the course of this investigation.

#### MATERIAL

Three different "wild" strains (*i.e.*, laboratory strains descended from wild flies) of *S. reynoldsi* and four different "wild" strains of *S. ocellaris* were available for this study. All the *S. reynoldsi* flies collected thus far have proved to be "bisexual"; that is, single females give both male and female offspring. On the other hand, "unisexual" as well as "bisexual" strains of *S. ocellaris* have been found. Females from the "unisexual" strains give male or female families. "Exceptional" males or females may be found in "unisexual" families. It might be pointed out that certain "bisexual" lines have been developed by selection from "unisexual" strains. The "bisexual" and "unisexual" strains of *S. ocellaris* are interfertile.

The metaphase chromosomes described and figured in this paper are germ-like chromosomes obtained from the gonads of 4- to 6- day old larvae (*i.e.*, 4 to 6 days after hatching of the egg at 74° F). At this stage of development the process of chromosome elimination characteristic of *Sciara* has occurred; consequently, the germ-like complement in both sexes is composed of four pairs of chromosomes. In the 4- to 6- day old larva the germ cells undergo a series of rapid mitoses. This period of mitotic activity is of somewhat longer duration in the testis than in the ovary.

In making this study, the aceto-carmin smear technique was found to give excellent results. The accompanying illustrations were all made from such preparations.

#### OBSERVATIONS

(a). *S. reynoldsi*. The metaphase group in the three wild strains of this species consists of two pairs of V-shaped chromosomes and two pairs of rods. One pair of rods is only slightly

longer than the other pair, while the larger pair of V's is nearly twice the size of the small V's. As explained below, it is clear that both pairs of V's are autosomes and that the sex chromosomes are a pair of rods.

(b). *S. ocellaris*—"bisexual." The two "bisexual" strains of *S. ocellaris* have a metaphase group like that of *S. reynoldsi* just described (Fig. 1, c). Aceto-carminic smears of brains from

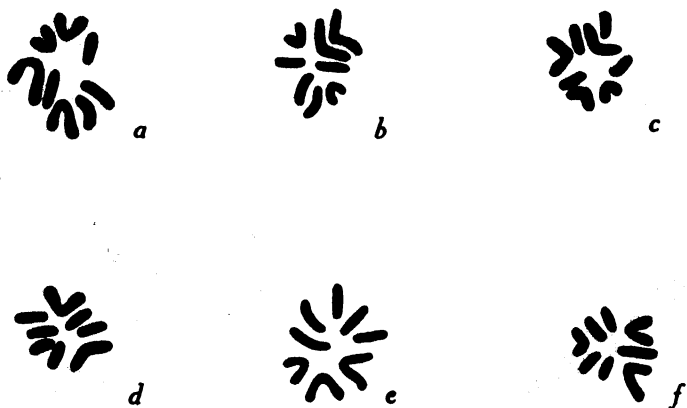


FIG. 1. Metaphase figures from larval gonads. Camera lucida drawings made with 1.4 mm objective and 15 $\times$  ocular at table level. Reduced one fourth.

a, b—*S. reynoldsi*, figures from two different wild strains.

c—*S. ocellaris*, figure from "bisexual" strain.

d—*S. ocellaris*, figure from "unisexual" strain.

e, f—*S. ocellaris*, F<sub>1</sub> from the cross of the 2-V strain by the 4-V strain. This 3-V configuration is found also in "unisexual" stock cultures (see text).

male larvae of "bisexual" *S. ocellaris* show seven chromosomes—the missing chromosome being one of the longer rods. Since in the male soma of *Sciara* only the maternal sex chromosome is normally present, it seems clear that the X is rod-like here and that the V's are all autosomes. This condition differs from that found in *S. pauciseta*, where the X is apparently V-shaped (Schmuck, 1934).

(c). *S. ocellaris*—"unisexual." In "unisexual" strains of *S. ocellaris* two different metaphase configurations are found. There are either three pairs of rods and one pair of large V's (Fig. 1, d; Fig. 2, c) or two pairs of rods, one pair of large V's and a single small V and rod (Fig. 1, e, f).

(d). *S. ocellaris*— $F_1$  from cross of the 2-V strain by the 4-V strain. Since the “unisexual” and “bisexual” strains of *S. ocellaris* are interfertile, the metaphase configuration in the  $F_1$  larvae from a 2-V female by a 4-V male (reciprocal cross made also) was determined. Here, as expected, three V’s and five rods are found, including one pair of large V’s, two pairs of rods and a single small V and rod. (See *e* and *f* of Fig. 1 and *d* of Fig. 2.) It is to be noted that the single rod in this metaphase configuration appears to be of the same length as the small V chromosome. At first it was supposed that the small V had originated from the rod, or *vice versa*, through the occurrence of a large inversion.



FIG. 2. Photographs of metaphase groups in larval gonads. 1750X.

a—*S. reynoldsi*.

b—*S. ocellaris*, figure from “bisexual” strain.

c—*S. ocellaris*, figure from “unisexual” strain.

d—*S. ocellaris*,  $F_1$  from the cross of the 2-V strain by the 4-V strain.

Examination of the salivary gland chromosomes of these 3-V larvae, however, revealed no such inversion; instead, essentially normal, synapsed chromosomes were found. Presumably, therefore, the small V arose from the rod, or *vice versa*, through translocation of the spindle fiber locus. The finer cytological details here have not yet been worked out.

#### DISCUSSION

Certain unpublished data of Mr. C. B. Davidheiser suggest that the genetic determination of “bisexuality” and “unisexuality” in *S. ocellaris* is autosomal rather than sex-linked. Since the heteromorphic chromosomes are autosomes and since all the “bisexual” strains of both *S. ocellaris* and *S. reynoldsi* investigated to date show the same 4-V, 4-rod configuration, while neither of the “unisexual” strains of *S. ocellaris* shows this configuration, it seems possible that the chromosome pair under consideration may be responsible for this determination. The



occurrence and origin of the 3-V, 5-rod configuration found in "unisexual" stock cultures of *S. ocellaris* have yet to be studied in detail.

#### SUMMARY

(1) In *S. ocellaris* two types of chromosome group are found: four V's and four rods in the "bisexual" strains and two V's and six rods in the "unisexual" strains.

(2) In the closely related species, *S. reynoldsi*, only the four-V, four-rod group has been found. All the strains of this species collected to date are "bisexual."

(3) In both *S. ocellaris* and *S. reynoldsi* the two pairs of V's are autosomes, while the sex chromosome pair is rod-like.

(4) The "bisexual" and "unisexual" strains of *S. ocellaris* are interfertile. By crossing the 2-V and 4-V strains of this species, an  $F_1$  generation showing three V-shaped chromosomes and five rods can be obtained. The salivary gland chromosomes of these  $F_1$  larvae give no indication of the origin of the small V through a large inversion in the rod, or *vice versa*. Presumably, therefore, the small V arose from the rod, or *vice versa*, through translocation of the spindle fiber locus.

(5) The genetic significance of the heteromorphic chromosome pair in *S. ocellaris* and its relationship to the determination of "bisexuality" and "unisexuality" in this species has yet to be investigated.

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## THE CELL THEORY ITS PAST, PRESENT AND FUTURE

EDITED BY JOSEPH MAYER

SECRETARY, SECTION L, AMERICAN ASSOCIATION FOR THE ADVANCEMENT  
OF SCIENCE

AMONG the important meetings held by the American Association for the Advancement of Science at Richmond, Virginia, at the end of December, 1938, were those having to do with the cell theory—its development, present status and future possibilities. These meetings were in the nature of a symposium and combined the best thought of the three sections of the association (on history, botany and zoology) that collaborated in the program.

Seven outstanding papers by eminent scholars were presented in the two sessions, in the morning and afternoon of December 27. Those taking part were: Professor L. L. Woodruff, of Yale University; Professor J. S. Karling, of Columbia University; Professor E. G. Conklin, of Princeton University; Professor G. A. Baitsell, of Yale University; Professor Paul Weiss, of the University of Chicago; Professor Franz Schrader, of Columbia University; and Professor C. E. McClung, of the University of Pennsylvania. The present writer was called upon to organize the meetings on behalf of the three sections concerned and to carry out the easily fulfilled duties of editor.

The morning session, which was devoted to historical aspects, brought some surprising results, especially as bearing upon the two men, Schleiden and Schwann, who until the present day have generally been given most credit

with respect to the origin of the cell theory, which they were supposed to have enunciated about a century ago.

There is agreement in the papers presented by Professors Karling and Conklin that the cell theory was projected sometime prior to the appearance of the works of Schleiden and Schwann, that these two men added nothing either in content or in clarity with respect to the theory as such, and that they in fact lent support to a general view of cell formation which was completely erroneous.

Professor Woodruff deals with the preceding period and with the influence of the microscope. One hundred and seventy years before 1838 Robert Hooke had described little boxes or cells seen under the microscope, and from his day onward other significant observations on cells had been recorded by such men as Leeuwenhoek, Malpighi, Grew, Swammerdam and Wolff.

With the beginning of the nineteenth century, the cell theory came into sharper focus and received fairly clear and explicit delineation at the hands of Mirbel (1802 to 1809), Detrochet (1824), Turpin (1826), Meyen (1828 to 1838), Brown (1831), Demortier (1832), Purkinje (1835), Mohl (1835 to 1838) and Valentin (1838).

All these significant contributions preceded the works of Schleiden and Schwann. Why, then, it is asked, have these two men been called the founders of the cell theory? Why the amazing situation "that we still continue to call it after them"? It would seem, as suggested by Professors Karling and Conklin, that bluff and brag on Schleiden's part entered very largely into the picture. He underestimated, ignored or ridiculed really important contributions of predecessors and contemporaries and thus gained a wholly unwarranted recognition. And Schwann borrowed from Schleiden's arrogant claims as to plant cells and applied the same views to animal cells. As Professor Conklin concludes: "It would be more accurate as well as more becoming to strike out of our literature these personal possession tags attached to important discoveries, such as . . . 'the cell theory of Schleiden and Schwann.'"

The afternoon session of the symposium, devoted to the present and the future of the cell theory, developed what appears like a cleavage in points of view between those who see the problem primarily in terms of physical forces (of surface tensions, physical pressures and electrical attractions and repulsions) and those who hold that living matter exhibits certain characteristics (such as variability, selective direction and unfoldment in a temporal sequence) which sharply differentiate the organic from the inorganic.

It is generally agreed that the organization of the cell is exceedingly complex and that there is still much to be learned about it. Yet, on the one hand, as Professor Baitzell maintains, the difference between the organic and the inorganic is "not one of *kind* but merely of *degree of complexity*. . . . Since the same materials are used in both domains, they must conform to the same elemental patterns." Recent advances in cellular knowledge are in fact due primarily to the work of physicists and chemists. Professor Schrader, who is concerned chiefly with the present status of mitosis, sponsors the renewed consideration of a "dynamic" hypothesis. He suggests, however, that such a hypothesis meets with many difficulties, which recent findings have by no means mitigated, and that it is a foregone conclusion that the final explanation will not be as simple as had once been thought.

On the other hand, it is pointed out by Professor Weiss that, although "the cells derived from an egg have definite, innate capacities of their own . . . the fact that the individual cell can differentiate in a variety of directions but actually differentiates only in one, calls for factors which direct each cell selectively into its proper course. These factors, by their very nature, are super-cellular." They apparently derive from the organism as a whole and suggest the presence of what Professor McClung designates as "racial material in a linear order within the chromosomes. . . . Since living systems have unique phenomena of a higher order (than the non-living), like reproduction, metabolism and consciousness, it is only

logical to conclude that there must be units of a new order to explain them."

The participants in the afternoon meeting are in agreement that there is no sharp break between the living and the non-living. The progressive series of integrations does not stop at the molecular but continues to higher orders. Furthermore, the chemical elements found in the living orders and their physical and chemical properties and interactions are, it would seem, precisely the same as those found in the non-living orders. If there is difference of opinion it appears to be as to whether the integrations of a higher order (such as the cellular) can be completely explained in terms of principles derived from a lower order (such as the molecular) or whether, since the living order has properties not found in the non-living, it must have its own peculiar units and be explained primarily in terms of those units. The future, it is held, should soon bring us closer to a resolution of such disputed questions.

These most interesting and important papers are presented in the pages that follow, with very slight omissions here and there to avoid needless repetition.

# MICROSCOPY BEFORE THE NINETEENTH CENTURY

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Each adds a little to our knowledge of Nature, and from all the facts assembled arises grandeur.—*Aristotle*.

ON this centenary of the formulation of the cell theory, it appears meet and proper to take a passing glance at the pioneers in microscopy, because, in truth, "the succession of men during the course of many centuries should be considered as one and the same man who exists always and learns continuously." The real value of an individual's work can be appraised with accuracy only when it is projected against the background of the intellectual life of his time. Schleiden and Schwann were indeed heirs of the past as well as outstanding examples of the fact that "the man and the moment must agree" if far-reaching results are quickly to follow. The cell theory initiated a rejuvenation of nearly all the chief branches of biological science, until to-day, as Lillie has recently emphasized, the cell has become "a sort of half-way house through which biological problems must pass, going or coming, before they complete their destiny."

The macroscopical anatomical techniques were the sole resort of naturalists until magnification was put into their hands to become the outstanding technique that actually created biological science—reduced the botanical and the zoological fields to a common denominator, the cell. As Sachs (1890) has, in effect, said,

the use of magnifying glasses taught those who used them to see scientifically and exactly. In arming the eye with these increased powers the attention was concentrated on definite points in the object, and observation had to be accompanied by conscious and intense reflection, in order to make the object, which is observed in part only by the microscope, clear to the mental eye in all the relations of the parts to one another and to the whole. Therefore, in marked contrast with the extremely slow progress in obtaining a mental mastery over the macroscopic morphological features of plants and animals is the work of the early students with the microscope.

Although the science of optics formally starts with Euclid, apparently what may be regarded as the earliest observations involving magnification are those of Seneca, who, during the first century of the Christian era, noted that small letters appear relatively large and clear when viewed through a glass globe filled with water; a result he attributed to the nature of the medium rather than to the curvature of the surface. Moreover, it appears that several Arabian opticians and other medieval savants were familiar with some of the properties of curved reflecting surfaces afforded by hemispheres and spheres of glass, but there seems to be no evidence that the possibilities of a lens as an optical instrument were appreciated until near the close of the thirteenth century. Roger Bacon indeed says: "If one views letters or any minute object through a lesser segment of a sphere of crystal or glass or other transparent substance, whose plane base is laid upon them, they will appear much larger and better. . . . Such an instrument is useful to old persons and to those with weak eyes." However, Bacon made no significant advance in the theory or the use of lenses, though possibly it is true, as suggested by Singer, "that groping with the instinct of genius, he did vaguely foresee both telescope and compound microscope."

The actual invention of convex spectacles probably did not occur until shortly after Bacon's death, and flourished when polishing lenses by a revolving lap was superseded by grinding with a spherical tool having the same curvature as that of the desired lens. The value of spectacles, apparently first appreciated by the illuminators of manuscripts, gradually filtered through to the populace, while the optical properties of lenses were studied by many, including Leonardo da Vinci, Leonard Digges, and Francesco Maurolico who reputedly was the first to use the theory of glass lenses to explain the operation of the lens of the eye. Moreover, Gianbattista della Porta stated in 1589 that "if you know how to combine the two kinds (con-

vex and concave lenses) properly you will see both things afar off and things neer hand, both greater and clearer"—one of several contemporary suggestions of some sort of bilenticular system.

Although the enthusiasm for optics at this period was largely motivated by a desire to improve spectacle lenses, concurrently occurred the first feeble impact of lenses upon biology if one may judge by the account of the itchmite by Thomas Mouffet in 1589 (published posthumously in 1634) and by figures of magnified organisms from the hands of George Hoefnagel in 1592 and Fabio Colonna in 1606. But even this was premature because the actual stimulation of investigation necessarily awaited more efficient lenses of very short focus and high magnifying power, as well as the effective discovery that two lenses can be adjusted in a system with still greater potentialities. The latter was accomplished during the fading years of the sixteenth century, apparently first by Dutch spectacle-makers, Hans and Zacharias Jansen, who placed, perhaps accidentally, two convex lenses in proper relative positions in a tube so that they acted as a true compound microscope, the image formed by the objective being magnified by the ocular before reaching the eye. Before long the invention came to the attention of Galileo who thereupon, in 1609, says he discovered the principle involved by a process of reasoning. However, the Galilean instrument gave no intermediate image and so was not a compound microscope according to the usual modern definition—a "true" compound microscope not reaching Galileo until 1624, and then indirectly from the Jansens.

At all events, the significant fact is that to Italian skies belongs the honor of reaping the first fruits of the use of a bilenticular system. Galileo in 1610 effectively employed the instrument and made the first recorded observations which during the following two decades encouraged microscopical studies by his associates in that brilliant coterie of scholars, led by Federigo Cesi, Duke of Aqua-



sparta, who met under the title of the Accademia dei Lincei. Prominent among the members were the botanist Colonna, the naturalist and scholar Stelluti, the astronomer Fontana, the Papal physician Faber and, until his death in 1615, the versatile sage and optician, Porta. Unfortunately few of their observations have reached posterity, other than those of Cesi on the "seeds" of ferns that entitle him to be regarded as the discoverer of spores, and those of Francesco Stelluti on bees, with the first published figures made with a compound microscope. (Fig. 1.)

Simple lenses were, of course, in more general use. It will be recalled that William Harvey in his classic of 1628 noted that he had seen with his *perspicillum* the beating heart of wasps, hornets and flies. So it was from these small beginnings with lenses, singly and in combination, that the urge for magnification in the biological field crept over western Europe, until toward the middle of the seventeenth century many were turning their hands to improving the compound microscope and their eyes to sporadic observing.

Probably the most significant gleanings of this period are in the works of the Sicilian Gianbattista Hodierna and the Neapolitan Francisco Fontana. The former published, in 1644, an investigation on the eyes of about thirty species of insects, and the latter, in 1656, miscellaneous observations including some on mites and several insects. And at the same time the encyclopedic Athanasius Kircher exploited the use of the microscope. He says, "Numbers of things might be discovered . . . facts hitherto unknown by all medical men and investigated by none of them," but he himself gave no detailed descriptions or figures of anything that magnification had revealed to him. However, during the next decade the gifted physician of Paris, Pierre Borel, was using the microscope to study a wide variety of objects and perhaps had a glimpse of human red blood cells. In 1656 he published the first volume devoted solely to microscopy. Ac-

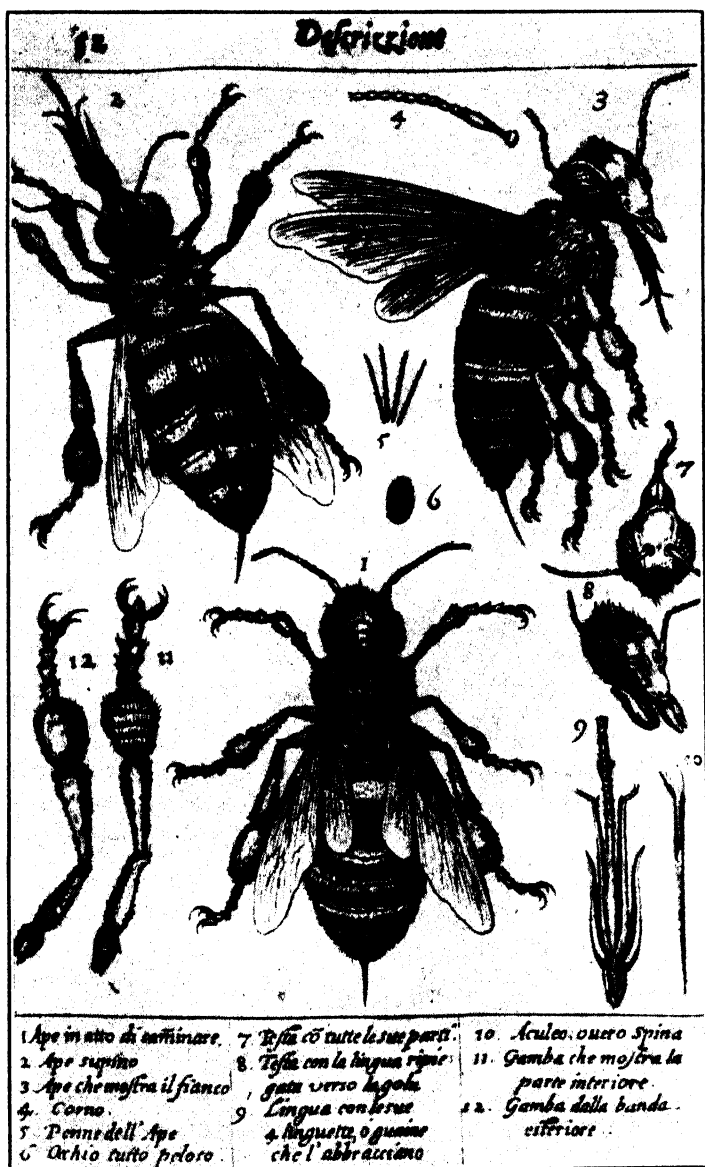


FIG. 1. Figures of Bees by Francesco Stelluti, first published in 1625. From "Descrizione dell' Ape," in Stelluti's "Persio Tradotto," Rome, 1630.

according to Singer, he records minute markings on young leaves which probably were outlines of cells, and not only saw stomata but also realized their power of opening and closing. And he describes what perhaps was protoplasmic movement. Moreover, apparently he was the first not only to turn the microscope on the early stages of the developing chick, but also to put the instrument to practical use in his profession—he was able to see ingrowing eyelashes and relieve a patient—the first use of the microscope in the field of medicine. (Singer, 1914, 1915; Torrey, 1938.)

But it appears that an Englishman, Robert Hooke, was the first to realize to the full the importance of studying nature with instruments which increase the powers of the senses in general and of vision in particular, and to convincingly express it in a most remarkable book published with the imprint of the Royal Society of London in 1665. Hooke (1665a) called his treatise “*The Micrographia; or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses and Enquiries Thereupon,*” and emphasized the need to supply the

infirmities (of the senses) with Instruments, and, as it were, the adding of artificial Organs to the natural. . . . One of them has been of late years accomplished with prodigious benefit to all sorts of useful knowledge, by the invention of Optical Glasses. . . . By the help of Microscopes, there is nothing so small, as to escape our inquiry. . . . By this the Earth it self . . . shews quite a new thing to us, and in every little particle of its matter, we now behold almost as great a variety of Creatures, as we were able before to reckon up in the whole Universe it self. It seems not improbable, but by these helps the subtilty of the composition of bodies, the structure of their parts, the various texture of their matter, the instruments and manner of their inward motions, and all the other possible appearances of things, may come to be more fully discovered. . . .

This is not the place to evaluate the many contributions to science made by the author of the “*Micrographia*” nor indeed by the book itself because, as a contemporary reviewer remarked, “This book contains more than can be taken notice of in an extract.” But one observation, in particular, holds our attention. This is “*Observ. XVIII.*

Of the Schematisme or Texture of Cork, and of the Cells and Pores of some other such frothy Bodies." Here are described and emphasized for the first time the "little boxes or cells" of organic structure and the use of the word "cell" is, of course, responsible for its application to the protoplasmic units of modern biology. And as if Hooke sensed the importance of this observation, he selected it to illustrate his method of scientific inquiry in a later treatise. (Fig. 2.)

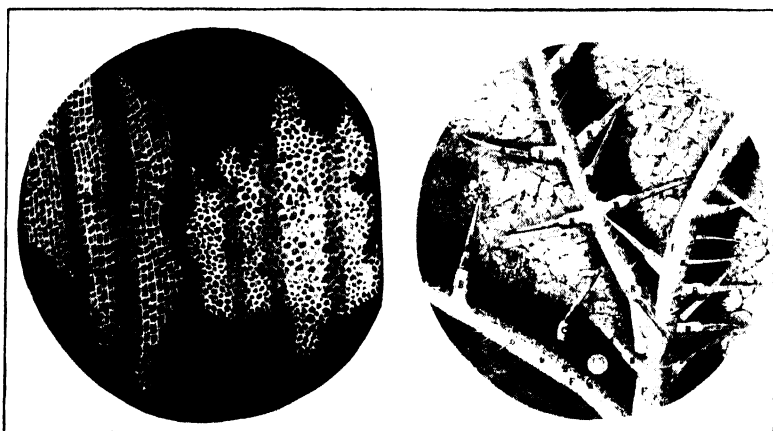


FIG. 2. Section of Cork, and Surface of Nettle Leaf. Hooke's "Micrographia," 1665.

The historic significance of Hooke's observations on cork (1665b) demands that he speak briefly for himself:

I took a good clear piece of Cork, and with a Pen-knife sharpen'd as keen as a razor, I cut a piece of it off, and thereby left the surface of it exceeding smooth, then examining it very diligently with a *Microscope*, me thought I could perceive it to appear a little porous; but I could not so plainly distinguish them, as to be sure that they were pores, much less what Figure they were of: But judging from the lightness and yielding quality of the Cork, that certainly the texture could not be so curious, but that possibly, if I could use some further diligence, I might find it to be discernable with a *Microscope*, I with the same sharp pen-knife, cut off from the former smooth surface an exceeding thin piece of it, and placing it on a black object Plate, because it was it self a white body, and casting the light on it with a deep *plano-convex* Glass, I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars.

First, in that it had a very little solid substance, in comparison of the empty cavity that was contain'd between. . . .

Next, in that these pores, or cells, were not very deep, but consisted of a great many little Boxes, separated out of one continued long pore, by certain *Diaphragms*. . . .

I no sooner discern'd these (which were indeed the first *microscopical* pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this) but me thought I had with the discovery of them, presently hinted to me the true and intelligible reason of all the *Phaenomena* of Cork. . . .

For . . . our *Microscope* informs us that the substance of Cork is altogether fill'd with Air, and that that Air is perfectly enclosed in little Boxes or Cells distinct from one another. . . .

Nor is this kind of Texture peculiar to Cork onely; for upon examination with my *Microscope*, I have found that the pith of an Elder, or almost any other Tree, the inner pulp or pith of the Cany hollow stalks of several other Vegetables: as of Fennel, Carrets, Daucus, Bur-docks, Teasels, Fearn, some kinds of Reeds, &c. have much such a kind of *Schematisme*, as I have lately shewn that of Cork. . . .

And then Hooke made significant observations which showed that some cells are not filled with air. He saw the sap and probably actually had a glimpse of protoplasm. He says; ". . . in several of those Vegetables, whil'st green, I have with my *Microscope*, plainly enough discover'd these Cells or Pores fill'd with juices, and by degrees sweating them out: as I have also observed in green Wood all those long *Microscopical* pores which appear in Char-coal perfectly empty of any thing but Air."

Again, a later study, "Of the stinging points and juice of Nettles, and some other venomous Plants" (Hooke, 1665c; Woodruff, 1919), is accompanied by a figure of the lower side of a nettle leaf in which the outlines of the epidermal cells are well delineated. And Miall (1912) remarks that "there is something very like a nucleus in one of them, but this may be accidental." However, Hooke did not emphasize any relationship between the structures he observed in the nettle and in cork.

With these few quotations we must leave the "Micro-graphia"—the first detailed and precise microscopical observations extant—with the thought that it had an immense influence, gleanings from its wealth of beautiful

plates “embellishing” technical and popular manuals on the microscope for a century and a half. While the book was being written, Samuel Pepys purchased a microscope and thought five pounds, ten shillings “a great price for a curious bauble.” And it is not recorded that Hooke’s demonstrations or those of the contemporary London physician, Henry Power (1664), changed his opinion, but the fact remains that from this time on magnification was established as a fundamental and indispensable aid in biological research. The so-called classical period in microscopy unfolds during the latter part of the seventeenth century with the outstanding contributions of Leeuwenhoek, Malpighi, Grew and Swammerdam. They literally created a new world and reversed the former conception that interest in objects is proportionate to their size.

The recluse of Delft, Antony van Leeuwenhoek, spent a long life in placid observations with simple microscopes made by his own hands. But his observations were exciting, for his lenses revealed a hitherto unseen world of living things—some so small that, as he says, “ten thousand of these living creatures could scarce equal the bulk of a coarse sand-grain.” His acumen and patience produced the longest and most important series of communications that a scientific society has ever received, extending over a period of more than fifty years. The last letter was addressed to the Royal Society of London from his death-bed in his ninety-first year.

Leeuwenhoek’s discovery of the Protozoa is described in a letter written in 1674. He says that in examining some pond water he observed “very many little animalcules, whereof some were roundish. . . . Others were somewhat longer than an oval, and these were very slow a-moving, and few in number. . . . And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that ’twas wonderful to see. . . .”

Such was his—and the world’s—first glimpse of animal-

cules, but the most famous "letter on the Protozoa" was penned in 1676. Thanks to a remarkably thorough study of Leeuwenhoek and his work recently made by Dobell (1932), we now have available for the first time not only the complete contents of this letter, but also a revised translation of the part originally published. The classic account of the discovery of *Vorticella* is still more informative in its new dress—the first description of an identifiable Protozoon.

So Leeuwenhoek put the Protozoa within the view of science, and then further on in this letter he did the same for Bacteria. In describing his first observations on pepper-water, he says: "The fourth sort of little animals, which drifted among the three sorts aforesaid, were incredibly small; nay, so small, in my sight, that I judged that even if 100 of these very wee animals lay stretched out one against another, they could not reach to the length of a grain of coarse sand. . . ."

Leeuwenhoek's observations were by no means confined to the world of "animalcules." He turned his lenses to the spinning glands of spiders, pupæ of ants, eggs of aphids, embryos of mussels, blood corpuscles and capillaries, the structure of muscle, transverse and longitudinal sections of many kinds of wood, and so on (Leeuwenhoek, 1722; Hooke, 1800–1807). And even to-day one marvels that Leeuwenhoek could see so much with his simple lenses. Although he was generous with his microscopes, his "particular method of observing" was persistently kept for himself alone. Dobell believes that Leeuwenhoek used some method of dark-ground illumination and this, in part at least, is almost surely the solution of his secret. (Fig. 3.)

Although Leeuwenhoek complained: "I oftentimes hear it said that I do but tell fairy-tales about the little animals," confirmation gradually came from Hooke, Bonanni, King, Harris, Gray and an anonymous contributor to the *Philosophical Transactions*, as well as from Christiaan Huy-



FIG. 3. Plant Tissues. Leeuwenhoek's "Anatomia seu Interiora Rerum, etc., 1687.



gens, though the important observations of the latter, transmitted to Leeuwenhoek in 1678, were not in print until recently (Hooke, 1678; Bonanni, 1691; King, 1693; Harris, 1696; Gray, 1696; Anon., 1703; Huygens, 1899). Then in 1718 appeared the first special treatise on animalcules in general and the Protozoa in particular by Louis Joblot of Paris. This is a remarkable book that describes new microscopes and many new organisms, and makes the first general attempt to give the latter appropriate names. Furthermore, in the study of the origin of the organisms Joblot was the first to boil infusions in order to eliminate life; a method exploited in studies on biogenesis by Spallanzani and others over a half century later without a thought of Joblot. It was Dr. John Hill, who, in 1752, gave the first formal classification of animalcules and, as he says, "arranged them into a regular method, and gave them denominations"—one, the familiar *Paramecium*. Then Linnaeus in the twelfth edition of his "*Systema Naturae*" grouped all of them under three genera. And finally O. F. Müller (1773, 1786) closed the century for animalcules by publishing the first extensive taxonomic monographs on these forms.

While these and other studies on microscopic organisms were enlivening every drop of water, concomitant progress was, of course, being made in revealing some of the main outlines of the finer structure of higher plants and animals under the initial stimulus of Leeuwenhoek and his contemporaries—Malpighi in Italy, Grew in England and Swammerdam in Holland.

Marcello Malpighi spent most of his life as professor of medicine at Bologna and almost continuously devoted himself to a varied program of investigations with the microscope. His versatility as well as his genius is shown in particular by his studies on the anatomy of plants, the function of leaves, the development of the plant embryo, the embryology of the chick, the anatomy of the silkworm and the structure of glands. Skilled in microscopic anat-

omy but with prime interest in the physiological aspects of organisms, his most significant contribution lies in his dependence upon the microscope for the solution of problems where structure and function, so to speak, merge, which is well illustrated by his ocular demonstration of the capillary circulation in the lungs. This was published in 1661, before Leeuwenhoek's similar studies, and is the initial discovery of prime importance made with the microscope because it completed Harvey's work on the circulation of the blood. Malpighi wrote: "I saw with mine eyes a truly great thing. . . . It is clear to the senses that the blood flowed away along tortuous vessels and was not poured into spaces, but was always contained within tubules, and that its dispersion is due to the multiple winding of the vessels." (Fig. 4.)

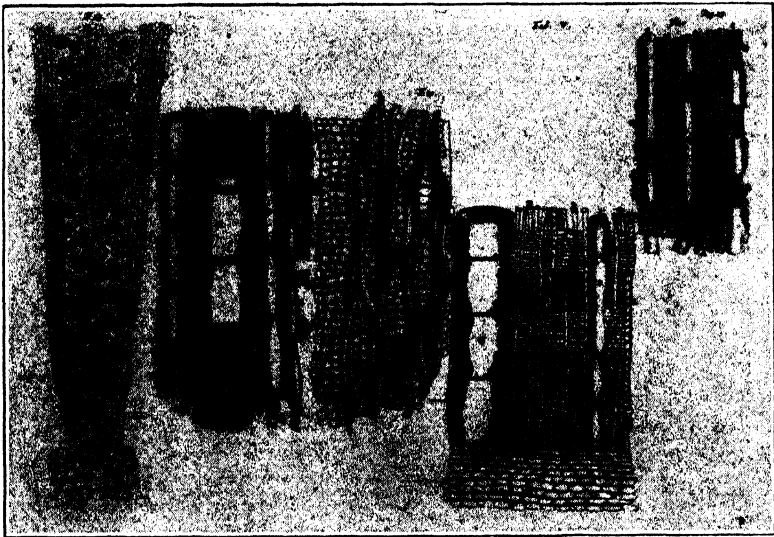


FIG. 4. Plant Tissues. Malpighi's "*Anatome Plantarum*," 1675-79.

Moving from Italy to England, we come upon the London physician, Nehemiah Grew (1672, 1682), who devoted most of his life to an intensive study of plant anatomy, and in this one field he paralleled, and perhaps surpassed, his Italian contemporary. Grew's work culminated in the

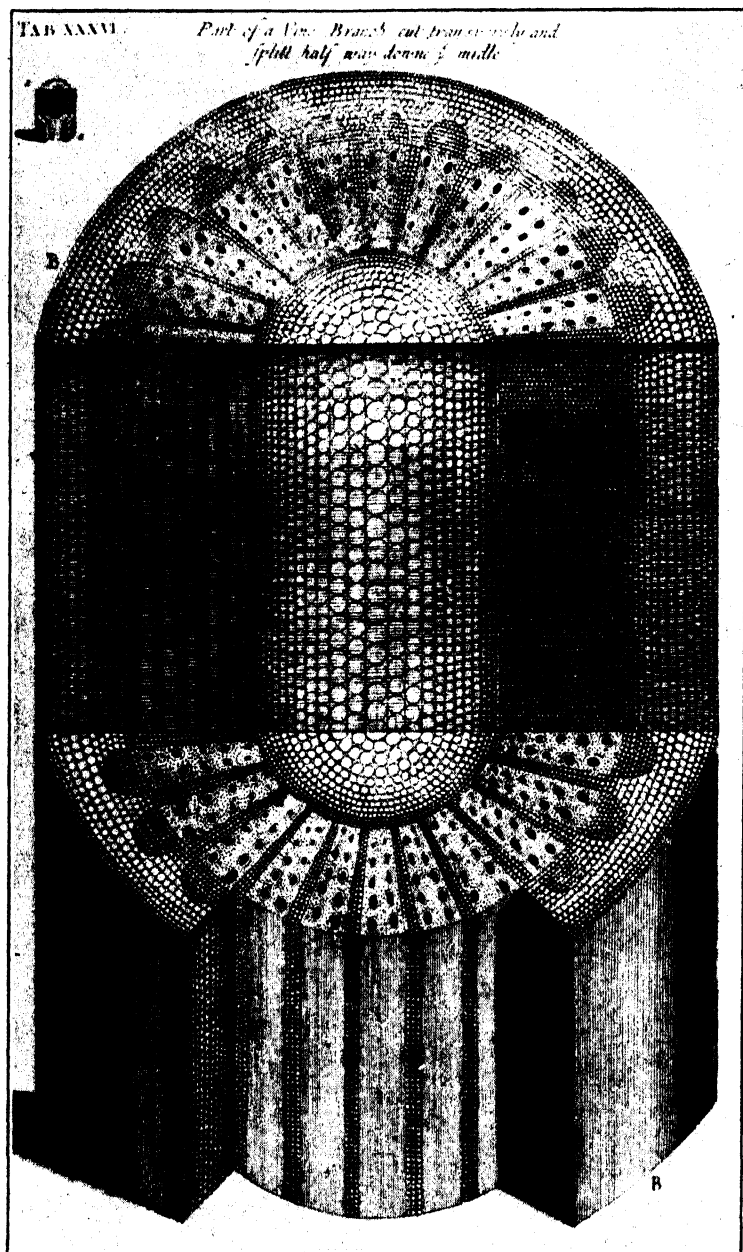


FIG. 5. Vine Branch. Grew's "Anatomy of Plants," 1682.

publication of his great "Anatomy of Plants" in 1682, several years after Malpighi's "Anatome Plantarum." The similarity of many of the observations unfortunately raised the question of Grew's indebtedness to Malpighi, but the implied slur is without the least foundation in fact. It was merely a case of two great pioneers working on essentially the same material at about the same time. Indeed, Grew voluntarily abandoned any claim to priority in regard to the discovery of vessels in plant stems and Malpighi undertook a Latin translation of Grew's work. (Fig. 5.)

Quite a different-tempered man was Jan Swammerdam, then at work in Holland on a magnificent series of animal dissections. By intensive application to exacting observations he ruined his health, but during his short lifetime he amassed sufficient material to support his chief thesis that the lower animals are as complexly constructed as the higher. Most of his observations were not published until over sixty years after his death, when they were collected in two splendid folios, profusely illustrated, under the title "Biblia Naturae" (Swammerdam, 1737-38; Hill, 1758). Some of the figures have never been excelled, and their range is wide, including, among others, the May fly, dragon fly, bee, ant, grasshopper, gall-insect, butterfly, hermit crab and tadpole. Swammerdam attained the last details visible with his single lenses; aided by a remarkable delicacy of manipulation of scalpel, forceps and injection technique. The work has been described, and probably justly, as the finest collection of microscopical observations ever produced by an investigator in this field. (Fig. 6.)

Strange to say, these pioneers with lenses, in particular Leeuwenhoek, Malpighi, Grew and Swammerdam, who created the golden age of microscopy, produced little immediate stimulation. This probably was in part due to the philosophically disturbing complexity of living things that overawed the naturalist, and to the fact that the pioneers collectively saw nearly all that was possible with the

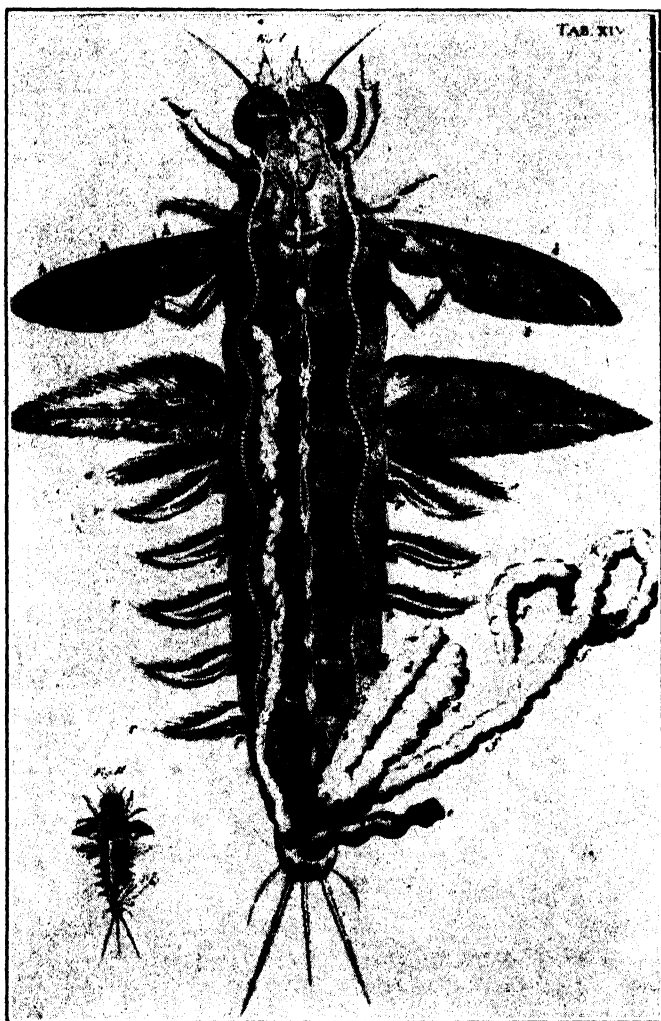


FIG. 6. May Fly, 1675, from Swammerdam's "Biblia Naturae," 1737-38.

available technique. The seeming finality of some of the work, such as that on the anatomy of plants, suggested the sterile notion that the subject was essentially exhausted. Indeed, it *was* exhausted for men of lesser capabilities. In general, what had been contributed to the stream of ideas that was destined a century and a half later to broaden out as the cell theory?

Malpighi, Grew and Leeuwenhoek were quite familiar with the elements that Hooke called cells. Malpighi referred to them as utricles, globules and saccules, and observed that their walls could be separated and the utricles isolated, so naturally he thought of the plant body in general as representing a union and coalescence of innumerable similar elements. Grew regarded the fundament of plant tissues as a fibrous "parenchymous" material with the fibers usually "woven and wound up" into an "infinite number of little cells or bladders" so that the whole is not unlike the "froth of beer" or a "piece of fine manchet." Vessels are formed by the confluence of "one single row or file of bladders evenly and perpendicularly piled." Leeuwenhoek described globules in the tissues of plants and animals. Thus he noted that leaves, with the exception of their vessels and fibers, are composed of globules which form not only a membrane on the surface but are variously placed and aggregated, and he discussed the nutritive supply of the globules with reference to the vessels. And Swammerdam, who was intent on organs rather than tissues, now and again mentions globules when using his higher lenses.

Naturally, as well as necessarily, the pioneers were more interested in what is now called histology than in cytology. They attempted to envisage the whole from the data at hand—and prematurely because, as it now appears, a sufficiently deep level of analysis had not been reached to render synthesis profitable. Thus Grew (1682a) in his "Anatomy of Plants" wrote:

The most unfeigned and proper resemblance we can at present make of the whole *Body of a Plant*, is to a piece of *fine Bone-Lace*, when the Women are working it upon the *Cushion*; for the *Pith*, *Insertions* and *Parenchyma* of the *Barque*, are all extream Fine and Perfect *Lace-Work*; the *Fibres* of the *Pith* running *Horizontally*, as do the *Threads* in a Piece of *Lace*; and bounding the several *Bladders* of the *Pith* and *Barque*; as the *Threds* do the several *Holes* of the *Lace*; and making up the *Insertions* without *Bladders*, or with very small ones, as the same *Threds* likewise do the *close Parts* of the *Lace*, which they call the *Cloth-Work*. And lastly, both the *Lignous* and *Aer-Vessels*, stand all *Perpendicular*, and so cross to the *Horizontal Fibres* of all the said *Parenchymous Parts*; even as in a piece of *Lace* upon the *Cushion*, the

*Pins* do to the *Threds*. The *Pins* being also conceived to be *Tubular*, and prolonged to any length; and the same *Lace-Work* to be wrought many Thousands of times over and over again, to any thickness or hight, according to the hight of any *Plant*. And this is the true *Texture* of a *Plant*; and the *general composure*, not only of a *Branch*, but of all other *Parts* from the *Seed* to the *Seed*.

And so the problem stood in general unchanged for upward of a hundred years. True, the eighteenth century produced a considerable number of students who turned their lenses on plant and animal structure, but none made impellingly significant observations except Caspar Friedrich Wolff (1759, 1768). Unlike his predecessors, he approached problems of the structure and development of organisms at a time when biologists were plagued by the doctrine of preformation, or "evolution" in the terminology of the period, and he planned his work to establish an inductive foundation for epigenesis in individual development. His famous doctoral dissertation, "*Theoria Generationis*," published in 1759 when he was twenty-six years of age, was the most important contribution since those of the classical microscopists, though it, as well as his later work, was almost completely disregarded by zoologists and botanists for some forty years. Its philosophical bearing was out of tune with those of the times, as represented, for example, by the ideas of Haller, the "abyss of learning" of the period. (Fig. 7.)

At the moment our interest in Wolff is not his insistence on epigenesis until it reached a *reductio ad absurdum* and tried to get something out of nothing, but the fact that he studied both plants and animals in a consistent effort to resolve their finer structure to a common denominator—to follow the process of development from the initial formation of tissue. Thus the embryonic parts first appear, he believed, as a transparent, gelatinous material which becomes saturated with a nutrient fluid. This sap is secreted in tiny droplets forming vesicles which gradually increase in size until as *Bläschen* or *Zellen* they become contiguous, except for the remnants of the substance be-

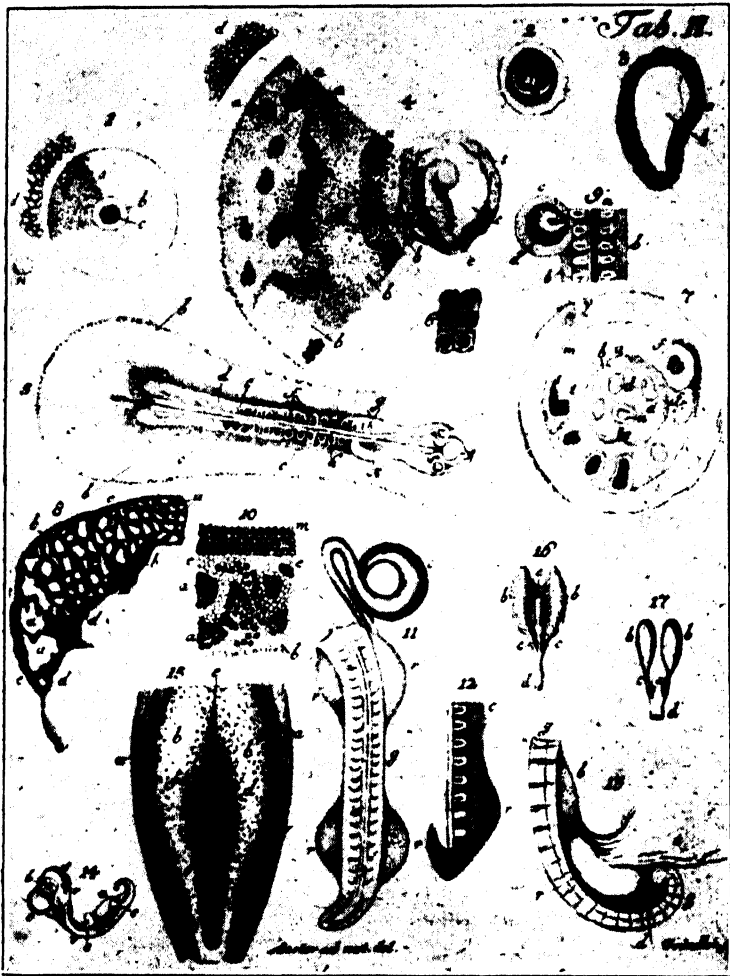


FIG. 7. Plate from Wolff's "Theoria Generationis," 1759.

tween, and so form what to-day we recognize as cells and cell walls. In general one is reminded of Grew's beer froth. Obviously, according to this scheme of cell formation the entities would be separated by a single lamina in a common matrix, though he believed there was communication between the cell cavities. And growth, he thought, takes place by the enlargement of existing cells as well as by the interpolation of new ones between the old. In brief,



the tissues of plants arise from one and the same fundamēt, variously modified.

Identical in principle according to Wolff is the underlying process of development in animals. He says that "the particles which constitute all animal organs in their earliest inception are little globules, which may always be distinguished under a microscope with moderate magnification." The formation of the intestine of the chick, for example, is from a relatively homogeneous substance by gradual differentiation, as in the case of the meristem in plants, and therefore the processes of nutrition and growth are essentially the same in both animals and plants. Doubtless influenced by his study of growing points in plants, he compared the genesis of animal organs to leaves or layers—the ancestor of the "germ layers" of a later day. Wolff undeniably laid the foundation for a broad understanding of the essential similarity of the formative elements of plants and animals, but this was not to be built upon until after the turn of the century.

Perhaps the most interesting contributions in the interim were Hill's "Construction of Timber," published in 1770, and Hedwig's "*De Fibrae Vegetabilis et Animalis Ortu*," dated 1789. Hill's sections of wood are not very highly magnified, but his figures and descriptions are in some cases quite good. That he approached as near as, but no nearer than his predecessors to a true concept of the basic structure is evident, for example, from his remark that in section "the corona is a ring usually more or less angulated in its outline, placed between the pith and the wood in all vegetables. The general circle is cellular, composed of blebs and vessels, as the bark and rind, and is perfectly of their nature; only that at different distances are disposed among it oblong clusters of different vessels." And in describing the pith of the rose, he says, "It has, in a slice of this thickness, the appearance of starry forms, with oval rays; but this illusion vanishes on cutting a thinner piece. When one is viewed of a thousandth part of an inch they appear only simple blebs." (Fig. 8.)

# CONSTRUCTION OF TIMBER,

From its EARLY GROWTH;

Explained by, the

## MICROSCOPE,

And proved from

### EXPERIMENTS,

In a great VARIETY of KINDS:

IN FIVE BOOKS.

On the PARTS of TREES; their VESSELS; and their  
ENCREASE by GROWTH: And on the different  
DISPOSITION of those PARTS in various KINDS;  
and the PARTICULARITIES in their VESSELS.

WITH FIGURES OF

Their various APPEARANCES; of the INSTRUMENT for  
cutting them; and of the MICROSCOPE thro' which  
they were viewed.

---

By JOHN HILL, M.D.

MEMBER of the IMPERIAL ACADEMY.

---

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And Sold by R. BALDWIN, in Pater-Noster-Row; J. RIDLEY,  
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M.DCC.LXX.

FIG. 8.

Hedwig's contribution, according to the interpretation of Sachs, shows careful observation though tinged with certain preconceived notions. His figures seem to be better than those of any of his predecessors. He saw cells aplenty, such as the epidermal cells of leaves, various parenchymatous tissues, and so on, but he regarded them all as vessels in the current indefinite sense of that term. And the record of the century should not be closed without mentioning Prochaska's "*De Structura Nervorum*," published in 1779, and Fontana's "*De Venin de la Vipere*,"

in 1781, both of which emphasized globules as important formative elements of the various tissues studied. But it is fair to say that these and other contributions, some of them "highly unimportant," gave no prophecy of the significance to be attained by cells during the dawning century.

Before leaving the development of this picture to others in this Symposium, we must consider the history of the microscope and microscopical technique which were silent but no less indispensable contributors to the formulation of the cell theory. "The history of the sciences," as Lillie (1938) remarks, "may be presented primarily as a history of ideas, but there would be no richness of ideas in science as we actually possess without richness in technique." Sometimes, to be sure, technique outruns ideas, and temporary sterility follows, but usually both surge ahead together. (Fig. 9.)

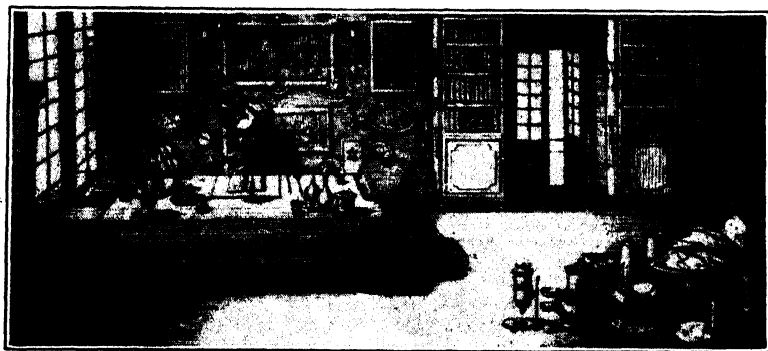


FIG. 9. An Early Eighteenth Century Laboratory. Joblot, 1718.

Leeuwenhoek, as we know, ground his own lenses and made his own simple microscopes, some 400 of them. In his hands the single lens reached *per saltum* the climax of its productivity, with in some cases a magnification of 200–300 diameters. This was while his contemporaries were depending chiefly upon compound microscopes, clumsy in construction and in most cases less efficient, though improvements on the type of instrument for which the term

“microscope” was coined by Giovanni Faber in 1625—a term that survived “smicroscope,” “engyscope,” etc. The chief contributions to the efficiency of the microscope during the period were made by Fontana (1646), Divini (c. 1648), Campani (c. 1660) and Hooke (1665) and gradually led to the ascendancy of a bilenticular system over the simple lens. Hooke is often credited with adding the field-lens to the ocular, but Monconys and Huygens, his contemporaries, preceded him in employing a plano-convex field-piece and a plano-convex eyepiece. At all events, the microscope remained for more than a century optically about as Hooke left it, while the rest of the instrument and its accessories advanced.

However, Bonanni (1691) knew much of what was needed, for he says he aimed at “an easy motion in examining the object; a convenient method of focussing; gradual focussing without the risk of losing sight of part of the object . . . ; an even illumination of the whole field; and a stable machine so that the eye can easily be replaced and see the object in the same place and light in order to draw it conveniently.” And a decade later James Wilson (1702), emphasizing technique, expressed the opinion that “the late improvements made by magnifying glasses are not so much owing to the making them and composing microscopes, as the methods of applying objects for the advantage of light.” Thus the microscope and its accessories at the beginning of the nineteenth century were born by the trials and errors of the eighteenth century instrument makers; in particular, John Marshall, a contemporary of Bonanni, and later Edmund Culpeper, Benjamin Martin and George Adams, Senior and Junior, won for English microscopes a high reputation. (Disney, Hill and Baker, 1928; Whipple, 1930; Clay and Court, 1932.)

The problems of chromatic and spherical aberration gradually became increasingly troublesome with higher magnification, involving lenses of shorter focal length and more nearly spherical form, but they were not successfully

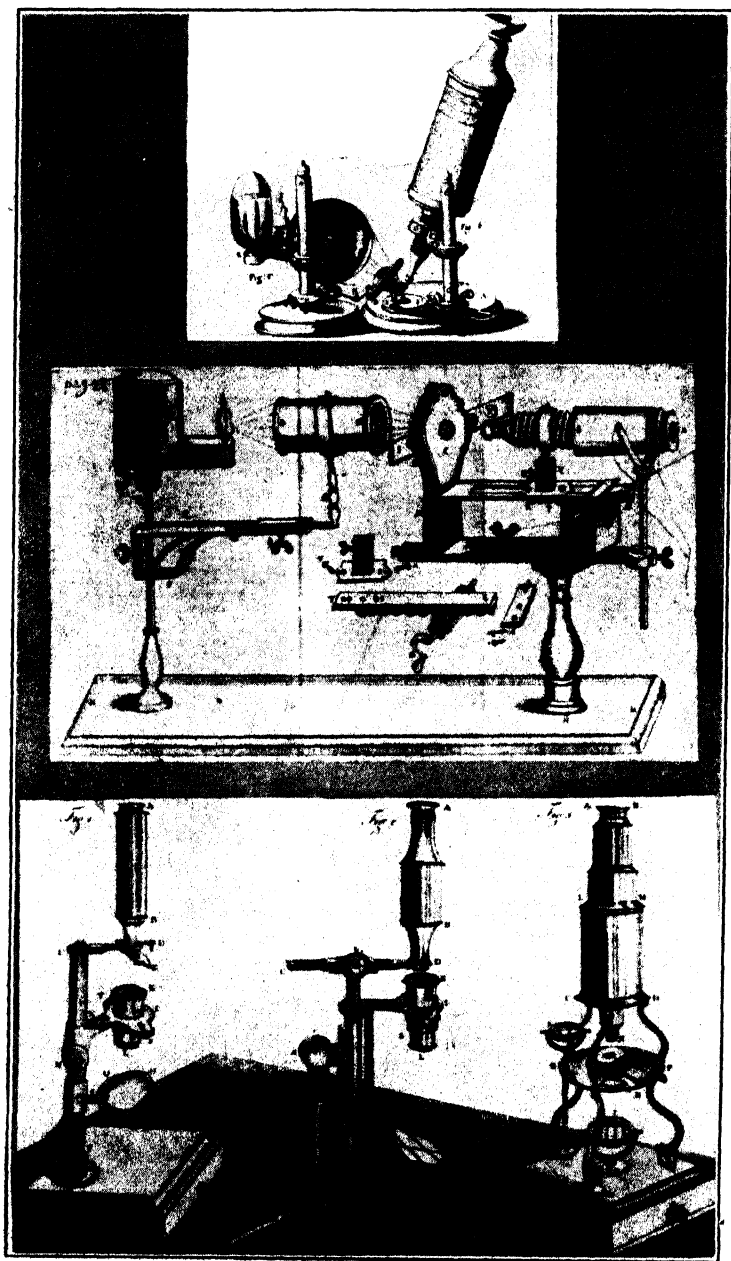


FIG. 10. Hooke's Microscope, 1665. Bonanni's Microscope, 1691. Types of Microscopes at the Close of the Eighteenth Century, Adams, 1787.

attacked until 1812 by Amici, who also apparently discovered the principle of the water immersion lens. In passing, however, it is interesting to note that Hooke (1678), while verifying Leeuwenhoek's animalcules, said, in effect, "that if you would have a microscope with one single refraction, and consequently capable of the greatest clearness and brightness, spread a little of the fluid to be examined on a glass plate, bring this under one of the globules, and then move it gently upward till the fluid touches and adheres to the globule."

The microscope itself, although the chief, was only one of many factors that contributed to the development of the technique of students of the "infinitely small." Methods for preparing objects to be examined played an indispensable part in unravelling the intricacies of the structure of plants and animals. It will be recalled that Hooke's success in resolving cork into cells was attained only when with "some further diligence" he cut off "an exceedingly thin piece of it"—probably the first reference to section cutting. Furthermore, Hooke later says that there are parts of animal and vegetable bodies "which cannot be well examined unless they be made to swim in a liquor proper and convenient for them," but if they "be put into a liquor, as water or very clear oyl, you may clearly see such a fabrick as is truly very admirable, and such as none hitherto hath discovered that ever I could meet with." This is apparently the first statement of the so-called wet preparation, a method that did not come into general use until the first quarter of the nineteenth century.

Leeuwenhoek, using simple microscopes, attached the specimen to a needle object-holder by glue, either directly or within a tiny glass tube in the case of liquids such as blood or infusion. "I did myself prepare," he says in 1674, "divers sorts of very slender, hollow glass pipes of which some were not thicker than a man's-hair." However, when occasion demanded, he used much larger tubes which he held by hand or clamped before the lens. Hooke

in his successful attempt to verify Leeuwenhoek's animalcules hit upon the basic principle of the transparent slide and coverglass preparation. "I take," he says, "instead of a glass pipe a very thin plate of Muscovy glass . . . and upon that I spread a very little of the liquor to be examined." And further, "All such bodies . . . whose surfaces are irregular . . . ought to be reduced to smoothness before they can be well examined." To accomplish this he put the material between two pieces of "very clear and thin looking-glass plate, very smooth and plain on both sides, and clean from foulness," and then pressed the preparation to make it very smooth and thin so that, for example, in the case of blood, no longer do "the multitude of those little globules confound and thicken the liquor so that one cannot perceive anything . . ."

Leeuwenhoek's other contemporaries using compound microscopes are reticent about their methods. To the best of my knowledge, Malpighi is silent on the subject, while Grew (1682b) merely states that it is necessary to study the anatomy of vegetables "by several ways of section, oblique, perpendicular, and transverse; all three being requisite, if not to observe, yet the better to comprehend, some things. And it will be convenient sometimes to break, tear, or otherwise to divide, without a section. Together with the knife it will be necessary to joyn the microscope; and to examine all the parts . . ."

Maceration of material, particularly plant tissues, one might suppose would have been hit upon early in preparing objects for the microscope, but it appears not to have been a generally recognized method before Moldenhawer in 1812, according to Sachs (1890), introduced this "important practical improvement" in technique. As a matter of fact, nearly a half century before, Hill had exploited maceration in his treatise on *The Construction of Timber*. Indeed, Benjamin Martin in 1742 suggested maceration to remove the epidermis of a leaf, but Hill used a much more elaborate method and first applied it to the

study of wood. Hill says that after several weeks' immersion, "by degrees, the parts loosen from one another; and, by gentle rubbing in a basin of water, just warm'd, they will be so far separated, that a pencil brush will perfect the business, and afford pieces of various size, pure, distinct, and clean."

Turning to the fixation and preservation of material for later study, again Hill (1770) was a pioneer. For example, he says:

Dissolve half an ounce of alum in two quarts of water; drop the pieces . . . for a few moments, into this solution; then dry them upon paper, and put them up in vials of spirit of wine. Nothing but spirit of wine can preserve their tender bodies; and till I found this method of hardening them first, that liquor often destroys them.

The origin of staining technique is difficult to determine, but if one follows Holzner, then Sarrabat in 1733 and Reichel in 1758 deserve priority. However, it seems not to be clear that they employed the dyes for microscopic purposes; merely observing the rise of solutions in twigs dipped into them (Smith, 1915a). But there is no question that Hill proceeded from maceration and fixing to true staining in his study of stems and thereby demonstrated structures otherwise invisible under the microscope. He used an alcoholic tincture of cochineal with considerable success and noted that since the stems of different species were not all stained the same, although the stain itself was the same, it must be "the construction of the body itself could in one instance have admitted it through passages which were closed to it in the other."

Equally interesting is another method described by Hill because it involved mordanting. He placed macerated twigs in a solution of sugar of lead and after two days transferred the material to a solution of quick lime, when, as he says, "the colourless impregnation . . . becomes a deep brown." Unless feeding Infusoria with colored material by Gleichen in 1778 and Ehrenberg in 1838 to show their internal structure is to be regarded as staining, there is apparently no further reference to such



technique until after the cell theory. Recently a botanist has justly stated that, "the great originality which John Hill showed in the manipulation of material . . . has not been given due credit" (Smith, 1915b; Conn, 1928, 1933.)

The modern microtome's history begins at least as early as Hooke's "penknife" and Leeuwenhoek's "sharp shaving razor," some of the sections cut with the latter being extant to-day. Before this it seems that no one had thought of section cutting, so all objects, unless naturally transparent, were viewed by reflected light. And little improvement in the tool was made until over a century later when "cutting engines" were developed to supply still thinner sections. One of the earliest machines was devised by Cummings and exploited by Hill in his study of wood. The invention has been ascribed to the Senior Adams, the well-known London maker of scientific instruments, but he does not mention it in the fourth edition of his "*Micrographia Illustrata*" that appeared a year later, in 1771. At all events, from this period on some sort of an "engine" was available for cutting sections as thin as "the two thousandth part of an inch," chiefly of plant tissue since embedding was of the distant future, but it was not until about 1860 that section cutting began to come into general favor with microscopists. (Fig. 11.)

Indeed, all microscopical technique was in its infancy not only during the period immediately preceding, but also long after that of Schleiden and Schwann. However, in spite of the backwardness of technique, the microscope itself had reached a degree of development that afforded unrealized potentialities. This is evident when we recall that, specifically with regard to the cell, botanists knew little more than that it consisted of a resistant wall with somewhat ill-defined contents, and zoologists with more difficult material were still more indefinite, although both were carrying on innumerable researches that threatened to swamp the science in minutiae. Unlimited complexity had supplanted apparent uniformity.

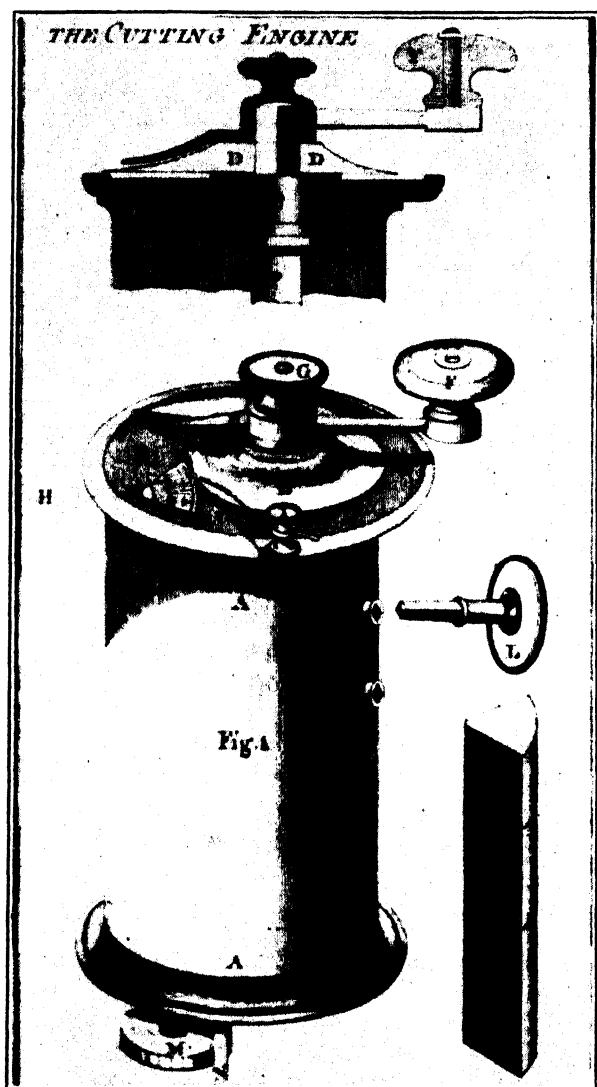


FIG. 11. Cummings' Cutting Engine. Hill's "Construction of Timber," 1770.

But the science was about to rise above the conceptions of Malpighi, Grew and Leeuwenhoek—Wolff was still unappreciated—and undergo the throes of a new birth. Just beginning were the significant investigations of Mirbel and

others, which during the following three decades laid the immediate foundations for—in fact, partly anticipated—the contributions of Schleiden and Schwann that were destined to pass the spark and precipitate the cell theory. It is not without significance that Huxley (1853), over ten years later under the impact of the theory, felt that Schleiden and Schwann had “grouped together an immense mass of details in a clear and perspicuous manner. Let us not be ungrateful for what they brought. If not absolutely true, it was the truest thing that had been done in biology for half a century.”

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## SCHLEIDEN'S CONTRIBUTION TO THE CELL THEORY

PROFESSOR JOHN S. KARLING

It is not an exaggeration to say that of all biological concepts none has proven more significant and fruitful of results than the cell theory which gradually emerged during the early part of the nineteenth century. Biologists are now agreed that the formulation of this doctrine marks an epoch in biological science and that in its far-reaching influence on research the cell concept merits a position beside the atomic theory and the doctrines of organic evolution and Mendelism. The enunciation of this concept and the realization that all plants and animals are composed of cells which are essentially alike in their make-up and formed in the same fundamental manner by division and that the activity of the organism is the sum total of the activities, interrelations and interactions of its individual cells, opened up horizons whose limits and possibilities have not yet been exhausted or even fully realized. The cell concept is the concept of life, its origin, its nature and its continuity.

Important and significant as the cell theory has proven to be, it is all the more a paradox of history that the two biologists who added little if anything new or original to this theory are now quite generally regarded as its founders. Nowhere is the paradox better illustrated than in the past, and current text-books of botany, zoology and biology which discuss the history of the cell theory. Of those which treat of this subject at all, 98 per cent. state almost unequivocally that Schleiden and Schwann originated the idea that all organisms are composed of cells which are formed in the same fundamental manner. This is also the belief expressed by the majority of text-books on cytology. Only two of the current texts, as far as I am aware, attempt to show that the cell concept was the cumulative result of a large number of investigations in the

early part of the nineteenth century. The belief that Schleiden and Schwann were the founders of the cell theory is so commonly taught in our high schools and universities that biology students are led to believe that, to use the words of Rich, "the idea sprang Minerva-like, fully formed and original from the substance" of their brains. Nothing could be farther from the truth, as a few of the more historically-minded biologists have pointed out from time to time.

It is thus proper and fitting on the occasion of this so-called "Centennial of the Cell Theory" that we refute again this erroneous conception and review anew the steps in the gradual development and emergence of the cell theory. What I shall here present on the relation of Schleiden to the cell theory is not essentially new and original. In 1879 and 1892 Hertwig reviewed to some degree the contributions of the early phytotomists and pointed out that the cell theory had its origin in study of plant anatomy. In 1907 Heidenhain gave a brief account of the development of the cell concept in his "Plasma und Zelle," while in the third editions of their text-books, Wilson and Sharp review the history of this subject at some length. Wilson, however, says, "Schleiden and Schwann are universally and rightly recognized as the founders of the cell theory." Gerould and Rich, in particular, have clearly emphasized the contributions of Dutrochet and pointed out that he formulated the cell concept a decade and a half before Schleiden and Schwann. It is also of interest to note here that the essential historical idea to be presented in this paper has been taught by Professor R. A. Harper for more than thirty years in his cytology classes at the University of Wisconsin and Columbia University.

Turning now particularly to Schleiden and the part which he played in the formulation of the cell concept of 1838-39, we can confine ourselves to his "Contributions to Phytogenesis," since this is his only paper which relates directly to the subject. In this contribution, which was

first communicated to Schwann in October, 1837, and published in Part II of Müller's "Archiv" in the following year, Schleiden makes these significant statements:

But every plant developed in any higher degree, is an aggregate of fully individualized, independent, separate beings, even the cells themselves. Each cell leads a double life: an independent one, pertaining to its own development alone, and another incidental, in so far as it has become an integral part of a plant. It is, however, easy to perceive that the vital process of the individual cells must form the very first, absolutely indispensable fundamental basis, both as regards vegetable physiology and comparative physiology in general. . . .

And further as relates to the origin of new cells, Schleiden proceeds at once to the wholly false idea of cell formation by a sort of crystallization about a granule. He says:

. . . Minute mucus granules are very soon developed in the gum, upon which the solution of gum, hitherto homogeneous, becomes clouded, or when a large quantity of granules is present, more opaque. Single, larger, more sharply defined granules next become apparent in the mass; and very soon afterwards the cytoblasts appear, looking like granulous coagulations around the granules. So soon as the cytoblasts have attained their full size, a delicate transparent vesicle rises upon their surface. This is the young cell, which at first represents a very thin segment of a sphere, the plane side of which is formed by the cytoblast, and the convex side by the young cell, which is placed upon it somewhat like a watch-glass upon a watch. . . . The entire cell then increases beyond the margin of the cytoblast, and quickly becomes so large that the latter at last merely appears as a small body enclosed in one of the side walls.

It is an altogether absolute law that every cell (setting aside the cambium for the present) must make its first appearance in the form of a very minute vesicle, and gradually expands to the size which it presents in the fully formed condition.<sup>1</sup>

A careful analysis of these statements shows that they include three fundamental premises, the first two of which are but briefly mentioned and implied, while the third is developed at great length. These premises are: first, *the recognition of the fact that plants throughout are composed of independent cells, which are the units of structure, physiology and organization*; secondly, *the duality of cells—their independent existence pertaining to their own development, and another as an integral part of the plant*;

<sup>1</sup> Translation by Smith.



and, thirdly, *that there is one general and common law of cell formation*. As has been noted above, the first of these premises is only incidentally mentioned by Schleiden. As to the second—the duality of the cell—it is but briefly stated. Historians, none the less, are inclined to credit the first tacit recognition of this fact to Schleiden. The third proposition relating to the law of cell formation is the main thesis and comprises all but a few pages of his contribution.

Having thus noted the fundamental propositions included in Schleiden's paper, and inasmuch as he does not give specific recognition to the discoveries of previous investigators, it is necessary that we make a critical survey of the literature prior to 1838 to determine whether or not these propositions are his own contributions to the cell concept. Many of the papers to be cited in this survey include much that is irrelevant and erroneous, but I shall refer only to that which relates directly to the development of the cell theory. Doubtless many fundamental contributions have been overlooked and omitted in this analysis, but sufficient literature has been examined and studied to indicate clearly Schleiden's relation to the cell theory. That plants are composed of cells had been known since the discoveries of Hooke, Grew, Malpighi, Leeuwenhoek and Hill in the seventeenth and eighteenth centuries, but it was not until the first quarter of the nineteenth century that the cell began to be regarded as the fundamental living individual unit as well as the structural element of all plant tissues. Mirbel and Sprengel (1802), Bernhardt and Link (1805) and Rudolphi (1807) recognized the cell as one of the units of structure in most plants, but they made a distinction between it and the elongated vessels present in the mature tissues. Sprengel at this early date, none the less, hints at the idea of a similarity in cellular structure of plants and animals when he says: "As far as I know at present . . . the nature of plants as well as animals appears to consist in the formation of a tissue, which we

can best compare to the cells of a honey comb, and, to be brief, call cellular tissue." He did not, however, regard the cell as a separate independent unit with a wall entirely its own. In 1806 Treviranus showed that elongated pitted vessels are formed by the disappearance of oblique cross walls, and in a like manner derived the true spiral vessels from long thin-walled cells. Thus was demonstrated for the first time that elongated vessels are also nothing but modified cells, but the significance of this discovery was not clearly realized until it had been confirmed by von Mohl in 1831 and Mirbel in 1833.

In 1802 Mirbel published his "*Traité d'anatomie et de physiologie Vegetales*," in which he states that, "Plants appear to be entirely composed of cells and of tubes, all parts of which are continuous." Like most phytotomists of that period, he made a clear distinction between cells and tubes, but in the second edition of his work in 1809 he omits tubes from the aphorism noted above. Mirbel more than any previous phytotomist insisted that all forms of plant structures are developed from cellular tissue. Simultaneous with Mirbel's second edition appeared Lamarck's (1809) "*Philosophie Zoologique*," in which he extends the concept of the cellular tissue as being the structural and organizing unit to both plants and animals, saying:

No body can possess life if its containing parts are not of cellular tissue, or formed by cellular tissue. Thus every living body is essentially a mass of cellular tissue in which more or less complex fluids move more or less rapidly; so that, if this body is very simple, that is, without special organs, it appears homogeneous, and presents nothing but cellular tissue containing fluids which move slowly within it; but if its organization is complex, all its organs without exception, as well as their most minute parts, are enveloped in cellular tissue, and even are essentially formed of it.

In the second volume he devotes a whole chapter to cellular tissue and adds further:

It has been recognized for a long time that the membranes that form the envelopes of the brain, of nerves, of vessels of all kinds, of glands, of viscera, of muscles and their fibers, and even the skin of the body, are in general the productions of cellular tissue. However, it does not appear that anyone has seen in this multitude of harmonizing facts anything but

the facts themselves; and no one so far as I know, has yet perceived that cellular tissue is the general matrix of all organization, and that without this tissue no living body would be able to exist nor could have been formed.

In a footnote to this chapter Lamarck states that he has taught this principle in his classes since 1796.

It is to be noted, however, that Mirbel and Lamarck did not regard the individual cell as the unit of structure and organization. To them, the continuous membranous cellular tissue rather than the cell is the elementary structure from which development proceeds. Lamarck, none the less, must be recognized as having pointed out once more the similarity of fundamental structure in plants and animals, although his interpretation may be somewhat incorrect.

Shortly afterward, Treviranus (1811) in a reply to Mirbel showed, as Malpighi (1675) and Link (1809) had done, that cells could be readily isolated as units by maceration, and particularly stressed the vesicular character of plant cells. This idea that the cell is an independent and individual unit as far as its own structure is concerned was confirmed by the studies of Moldenhawer in 1812 in which he employed the maceration technique very extensively. By this method he showed clearly that parenchyma cells and elongated vessels are closed sacs and tubes with walls of their own rather than open spaces in a membranous tissue.

These early studies may be said to have paved the way for the outstanding study and brilliant generalization of Dutrochet in 1824, who may well be called the forgotten man of the cell theory. By boiling plant and animal tissues in nitric acid and water, he isolated the cells as distinct entities with their own walls and contents, and this led him to the generalization that "the cell is the fundamental element of organization." In 1824 he published the little book entitled "*Anatomical and Physiological Researches on the Intimate Structure of Animals and Plants, and Their Motility*" in which we find as definite and clear an enunciation of the cell concept as that formulated in 1838

and in 1839. Inasmuch as Dutrochet will doubtless be discussed in detail by other speakers on this program, I shall confine myself largely to his botanical statements and their relation to Schleiden's ideas:

I must repeat here that which I have stated above regarding the organic texture of plants: we have seen that plants are composed entirely of cells, or of organs which are obviously derived from cells; we have seen that these cells are merely contiguous and adherent to each other by cohesion, but that they do not form a tissue actually continuous. . . . All the organic tissues of plants are made of cells, and observation has now demonstrated to us that the same is true of animals. . . . One can therefore draw the general conclusion that the globular corpuscles which make up all the organic tissues of animals are really globular cells of extreme smallness, which are united only by cohesion. Thus all the tissues, all the organs of animals are really only a cellular tissue diversely modified. This uniformity of ultimate structure proves that organs really differ from the other only in the nature of the substances which are contained in the vesicular cells of which they are composed.<sup>2</sup>

Here we have for the first time, as far as I am aware, a demonstration by observation of the lucky guess or hypothesis which Oken made in 1805 and 1810 when he said, "animals and plants are throughout nothing else than manifoldly divided or repeated vesicles." Dutrochet's confirmation of this hypothesis and his clear-cut expression that plants and animals throughout are composed of cells was made almost a decade and a half before Schleiden and when Schwann was but a lad of 14 years. While it is true that Dutrochet did not recognize as clearly as his successor Meyen that the cell has its own life cycle, that he failed to observe the nucleus, and had little knowledge of the inner structure of cells, he none-the-less recognized the independence and individuality of these units as well as their relation to the structure and organization of the plant and animal as a whole. His generalization as to the fundamental nature of all organisms becomes all the more impressive in comparison with those of Schleiden and Schwann when we consider the inadequacy of the microscope of 1824 and the fact that he did not have the excellent and meaty discoveries of Meyen, von Mohl, Brown, Müller, Valentin, Henle, Purkinje, and others to draw on. As one

<sup>2</sup> Translation by Rich.

reads Dutrochet's contribution more extensively, one can not escape the feeling that we are perhaps fifteen years too late in our "Centennial of the Cell Theory," and that future generations will regard this celebration as a humorous incident of the strange way in which science makes progress.

Dutrochet likewise recognized the cell as the physiological unit and thus antedates Meyen, von Mohl, Raspail, Schleiden and other phytotomists in this respect also. He adds:

It is within the cell that the secretion of the fluid peculiar to each organ is effected. . . . Thus the cell is the secreting organ par excellence. It secretes inside itself, substances which are, in some cases, destined to be transported to the outside of the body by the way of the excretory ducts, and, in other cases, destined to remain within the cell which has produced them. . . .

In this connection it is also well worth while to note another contribution of Dutrochet, a restatement of which brought Schleiden great renown fourteen years later as its originator. In his treatment of cell formation Schleiden discusses at great length the question as to what constitutes growth, and in conclusion he lays down three principles which are commonly taught to-day. Growth, to him, consists, first, of the formation of new cells; secondly, of the expansion or enlargement of cells; and thirdly, of a thickening or lignification of their walls. With his usual arrogance Schleiden announces these principles as his own by the statement "that, in respect to scientific botany, the idea (to) grow still requires a new foundation in order to be capable of being applied with certainty." With this in mind, let us now turn to Dutrochet. "Growth," he says, "results both from the increase in the volume of cells, and from the addition of new little cells. It is therefore, evident that new, rudimentary cells are formed, which, by increasing in size, finally become cells such as those which have preceded them in order of appearance and development." Dutrochet elaborated this principle further and discussed growth at great length in his extensive memoir in 1837, and if Schleiden had taken the trouble

to familiarize himself with the botanical literature of the time, he would have found his ideas concerning growth clearly expressed and elucidated. Perhaps he was familiar with Dutrochet's contribution but deliberately stated the latter's ideas as his own.

The individuality of the cell as a structural and physiological unit was again pointed out and emphasized in 1828 by Meyen in his booklet on "Anatomical-Physiological Researches on the Content of Plant Cells."

"The cells of plants," Meyen says, "are to be regarded therefore as single, independent individuals, small plants as it were in the larger, each of which carries on its own life independent of its surroundings. The mechanism, however, becomes more complex by their aggregation into groups, whereby the nature of the plant is heightened. . . . Cells are the organs of the plants which take up the raw (humors) substances for the nutrition of the same." Here we have the clearest expression so far of the conception that the cell is the unit of life—perhaps the only unit of life. The multicellular organism is an aggregate; its unity is due to the interrelations and interactions of cells. This concept of the organism as a mass of cells which integrate and interact to form a coordinate whole is perhaps the real climax of the cell theory.

Outside of its relation to the independence and individuality of the cell, this book by Meyen, written when he was but twenty-four years old, is particularly significant in relation to the more modern cell theory, because it deals almost exclusively with the content of the cell, to which latter was given the name protoplasm. While many of his ideas were incorrect, he nevertheless focused particular attention on the more viscid, vital elements of the cell rather than on the cell wall, and he is thus to be regarded as one of the forerunners of the protoplasm doctrine. A year earlier (1827) he had described and figured very accurately the nucleus in *Spirogyra princeps*, but the significance of this discovery was obscured by his fanciful belief that the nucleus gives rise to infusoria as the algal fila-

ments decay. In 1830 he published his text-book on phytotomy, in which occurs the oft-quoted passage relating to the cell:

Plants appear either singly so that each forms an individual as in some algae and fungi, or they are united in greater or smaller masses to form a more highly organized plant; even here each cell forms an independent isolated whole; it nourishes itself, it builds itself up, and elaborates the raw nutrient materials, which it takes up, into very different substances and structures.

Here we have a statement of the character and inter-relationship of the cell that is so true and modern that it may well have been uttered to-day. In it occurs not only the doctrine of the independence and individuality of the cell, but its duality as well. The cell, according to Meyen, not only leads an independent existence relating to its own life but plays an integral part in the organization and development of the whole plant. To Meyen, as we have already noted above, the higher plant or organism is but an aggregate or colony of individual cells—a statement which Schleiden and Schwann emphasized so strongly in their contribution, without acknowledging its authorship. It may well be noted here, however, that this idea itself is by no means original with Meyen. Twenty-three years earlier the naturalist and philosopher Lorenz Oken (1805) made the following statement as to the nature of plants and animals, which has already been noted above: “. . . Animals and plants are throughout nothing else than manifoldly divided or repeated vesicles.” Again in 1810 he says, “In so far as the plant is a multiplication of primitive vesicles, it consists of cellular tissue.” And finally several years later (1835) he amplifies this idea as follows: “The ground work of all plant and animal substances consists of delicate vesicles, . . . The lowest plants like the fungi . . . and algae . . . are nothing more than such vesicles which appear singly or grown together. The cellular tissue of plants is therefore nothing more than an aggregation of primitive plants. The same meaning applies to the cellular tissue of animals.” Turpin also had expressed this idea as early as 1825.

While it is true that Oken's statements were not based on observations and facts and seem to be nothing more than a fortunate guess or hypothesis, the idea is nevertheless clearly expressed, and he must be given serious consideration as foreshadowing the cell concepts expressed by Dutrochet and Meyen and later restated by Schleiden and Schwann.

Returning again to Meyen, the significance and value of his discoveries and generalizations are unfortunately obscured by his insistence that elongated vessels and laticiferous ducts were elementary organs in addition to cells. He retained this idea of the latex vessels as late as 1837, which leads one to suspect that even at this date he was not completely certain of the cell as a universal elementary structural unit.

This brief survey of the literature shows clearly that the first two premises of Schleiden—the doctrine of the cell as an independent, individual, structural and physiological unit, and its dual existence—had been clearly stated and demonstrated several times prior to 1838, and it is thus apparent that Schleiden added nothing new or original in the opening paragraphs of his "Contributions to Phytogenesis." It is true that he does not definitely claim these ideas are his own, but his failure to recognize the contributions of other investigators puts him at once in the class of the borrowers rather than that of the original investigator.

Turning now to the third and major premise of Schleiden's contribution—namely, that there is one "universal law for the formation of the vegetable cellular tissue in the Phanerogamia," we find that it is true only in statement and completely false as to the method of accomplishment. All biologists are now agreed that there is but one method of cell formation, but that it occurs by division of a preexisting cell—not by the aggregation of mucus granules in the cytoplasm to form first a nucleole and cytoblast and then the cell. This fantastic and completely erroneous idea of cell origin was regarded by Schleiden as the



most important and fundamental part of his contribution, and on this he laid the greatest stress. The first two propositions noted above were but introductory to this major premise.

If we examine again the literature closely in relation to the origin of cells, we find that Schleiden's false theory is not original but merely a modification of an old theory of the early phytotomists which was also rather widely held by zootomists of that period. At that time there were two outstanding views as to how cells arise: first, the view of the zootomists and phytotomists that the nucleus and cell develop from an aggregation and confluence of granules of various sorts in the viscid content of the cell; and secondly, the view of numerous phytotomists that new cells arise by division of a preexisting cell. None of these theories, however, had been extended to all plant and animal cells and formulated as a universal law of cell formation. The first view seems to have arisen from the old theory of Sprengel that new cells are formed by the expansion and vesiculation of granules and bodies in the cell. In 1802 Sprengel proposed this theory as follows from his microscopic study of bean seeds:

If you examine a bean before it germinates, you will observe in the hollow parts no specific form, no regular structure; one grain close by another. . . . The bean now germinates: the two cotyledons between which the embryo is enclosed swell: the skin loosens. If you take a very delicate, completely transparent section from the cotyledons you become aware of an aggregation of small vesicles intermingled with the moisture, which, in my opinion, can be called the true rudiments of the cellular structure. The more the plant expands the more regular, cell-like, and continuous this tissue becomes. . . . The delicate vesicles, which are yet swimming about in the moisture, appear to have the ability to become cells, and are perhaps in succession transformed into such. In a similar manner, you will note, all other plants originate from seeds. The irregular, unorganized chaos of dry seeds takes on a regular structure, while through the imbibition of moisture vesicles develop, which, crowded and expanded within and without by sap and confined by adjacent vesicles, assume a definite angular shape. . . .

These granules which Sprengel described as vesiculating to become cells are now recognized starch and aleurone grains. His theory in modified form was later adopted by Treviranus, Rudolphi and Kieser, and they extended it

to involve a wide variety of granules in the cell. Eventually, it appears to have been taken over by the zootomists, who further modified and adapted it to fit the more recently discovered data from animal tissues. Schleiden distinctly rejects Sprengel's theory, but after all, there is very little fundamental difference between the idea of cells originating by the swelling of starch grains and by the vesiculation of mucus granules or cytoblasts. I shall here refer to a few of the descriptions of cell origin in zoological literature to show the relation of these views to Schleiden's idea of cell formation.

In 1830 Baumgartner described the formation of red corpuscles by the aggregation and condensation of granules in the blood serum. In 1836 C. H. Schultze confirmed the observations of Baumgartner and described nuclear and cell formation in the blood of amphibia and fish as follows:

The true nuclei are formed in the following manner: the corpuscles which are becoming flattened always have in the beginning two or more yolk granules, or sometimes whole masses of smaller ones. Out of those are formed the nuclei, either by the fusion of numerous small ones to form a larger granular nucleus, or by the gradual disappearance of the smaller granules until a single large one remains. . . . On the other hand, in *Perca fluviatilis* and *Cyprinus erythrophthalmus* I find that the nuclei are more often the primary structures and are formed from the transformation of yolk granules, and that the lens-shape vesicles later arise around these.

In the same year Valentin (1836) figured the same type of cell formation in pigment cells, and in 1837 Wagner demonstrated its occurrence in the ovum. In these and the older contributions of the phytotomists seems to lie the kernel of Schleiden and Schwann's peculiar conception of nuclear formation in the cytoplasm and the subsequent development of cells around the cytoblast. Their figures of the process are so fundamentally similar to those of Schultze and Wagner that they may well have been based directly upon them. As a matter of fact, Schwann cites Wagner's figures in his supplement as a confirmation of his observations. Erroneous and fantastic as Schleiden's idea of cell origin in embryonic tissues was, he far sur-

passed it in imagination when he came to describe cell formation in the cambium of woody stems. Here he abandons his previous idea of cell formation and proposes a theory of simultaneous and spontaneous generation of cells from an unorganized fluid:

Here, so far as we are at present acquainted with the subject, there is no formation of cells within cells, here no expansion of all sides of the originally minute vesicle occurs, there is here no cytoblast upon which the young cell might be developed; but beneath the outermost layer of cells, which are comprised in the term bark, an organizable fluid is poured out, as it were, into a single, large intercellular space, which fluid, as it seems, consolidates quite suddenly throughout its entire extent into a new, altogether peculiarly-formed tissue of cells, which are deposited one upon another, the so-called prosenchyma. Here, moreover, there is decidedly no formation of vascular bundles from cells of lower order, for all of them originate simultaneously and of their full size; and what has been called (spiral) vessels of the wood, is something which differs immensely from the spiral vessels of herbaceous plants, both in respect of their origin, and probably of their physiological significance also.

That is Schleiden's idea of cell formation in the cambium and secondary thickening in woody plants—purely a hypothesis spun out of thin air.

How unfortunate for biological research of that decade and the reputation of Schwann that Schleiden did not choose the alternative and correct view of his contemporary botanists! It had been shown again and again before 1839 that new cells are formed by the division of preexisting ones. In 1830 Morren clearly described the origin of new cells by division in the alga, *Crucigenia*; Dumortier figured and described division again briefly in *Cladophora* in 1832, and in 1833 Mirbel observed its occurrence in the spores of *Marchantia*. In 1835 Winter, under the direction of von Mohl, published a long dissertation entitled "On the Multiplication of Plant Cells by Division," in which he showed for the first time the successive stages of cell division in *Cladophora*. Von Mohl republished this paper as his own in 1837 and again in his "Vermischte Schriften" in 1845 without mentioning Winter's name, which leads us to suspect that he made the original discovery and possibly wrote Winter's thesis. This paper

was followed in 1836 by another contribution by Morren on division in *Closterium*, and in 1837 he gave a brief summary of the discoveries of cell origin by division of a pre-existing cell in which he says that "the succession of these observations will suffice without doubt to establish this fact (cell origin by division) as one of the most established of vegetable organogenesis." In the same year Dumortier reported that the cellular tissue of the liver of molluscs and gastropods originated by division of preexisting cells in the same manner as he had described for *Cladophora* five years earlier. In 1838 Mohl described the origin of the guard cells of stomata by division, and in the same year Meyen reported the occurrence of cell division in *Scenedesmus* and the corticating cells of *Chara*. In this volume on plant physiology Meyen declared that cell division was a common occurrence in filamentous algae, fungi, and the Characeae. He followed this in 1839 with another paper on division in the embryo sac of *Viscum*, the spore mother cells of *Trichostylum*, the guard cell rudiments of stomata in *Hyacinth* and the vegetative cells of *Merismopedia* and *Ceramium*. In the same year Mohl described and figured in considerable detail division of the spore mother cells of *Anthoceros*, and showed the presence of fibers between the divided plastids which are now generally recognized as the rudiments of the achromatic spindle figure.

Numerous as these observations were, however, none of the investigators recognized the universality of cell division or formulated a general law of cell formation, or showed the relation of the nucleus to cytokinesis. There were at hand, none the less, numerous data, descriptions and figures on the correct method of cell origin which Schleiden and Schwann might have utilized. Schleiden, in his profound ignorance or disdain of the contributions of others, disregarded them completely, while Schwann, although apparently familiar with some of them, did not regard these discoveries as being of much significance.

The immediate effect of Schleiden's error was to place

a false emphasis on the erroneous distinction between endogenous or free cell formation and cell division, and it was not until after many years of study that microscopists realized that the two methods are fundamentally identical.

It is thus obvious from this survey that all the facts and hypotheses as well as misconceptions included in Schleiden's paper are to be found in biological literature prior to 1838 and that even unto the errors and inaccuracies he added nothing new or original. Schleiden's misleading theory nevertheless focused attention on the wide prevalence of nuclei in plant cells and their possible relation to cell formation, and this seems to be his primary contribution. It must be borne in mind, however, that his idea of this relationship was completely false and confusing and that he regarded the nucleus as being absorbed or disappearing after the new cell had been fully formed. The very enormity of his error coupled with the disdain he had for his contemporaries nevertheless stimulated others to study the nucleus with greater care, if for no other reason than to disprove Schleiden's theory. They were, consequently, led to the discovery that nuclear division usually precedes cell division. In the writer's opinion, therefore, it is doubtful that Mohl, Barry, Unger, Nägeli, Remak, Kölliker, Hofmeister and others would have directed their attention so soon to the relation of the nucleus to cell division had not Schleiden perpetrated his fantastic and erroneous idea. This error together with Schleiden's commanding position as a teacher proved thus to be a ferment of deep influence in revitalizing biological science. By their insistence on the study of the early developmental stages of tissues, Schleiden and Schwann brought about a marked change in attitude, and after 1838-1839 histology became very different from what it had been before. With the restatement and elaboration thus of Dutrochet's and Meyen's concepts of the cell in terms of the newly accumulated data, histologists, physiologists, pathologists and embryologists began to think primarily in terms of cells.

Does this critical appraisal of Schleiden's contribution

mean therefore that his name should be excluded from the cell theory and that Schwann stands alone as its founder? Certainly not. Except for his careful and accurate demonstration of the true cellular nature of numerous animal tissues, Schwann must stand or fall with Schleiden, because the most fundamental part of Schwann's theory—namely, that there is one fundamental principle of cell formation—was borrowed from Schleiden. All that Schwann knew and wrote in relation to the cellular structure of plants and the formation of cells came from Schleiden's paper, as he admits in his introduction. He adopted Schleiden's theory of cell origin completely, extended it to animal cells in which it had not previously been shown, and compared nuclear and cell formation to the development of crystals in a mother liquor. Schwann thus attempted to bridge, figuratively at least, the gap between the organic and inorganic. If we analyze further his interpretation and extension of Schleiden's theory, we find that it is also a repetition of the fundamental idea of Wolf's (1759) doctrine of generation, as Huxley (1853) has already pointed out. "The generation of cells," says Schwann, "takes place in a fluid, or in a structureless substance," the cytotblastema. Compare this with Wolf's statement:

Every organ is composed, at first, of a mass of clear viscous, nutritive fluid, which possesses no organization of any kind, but is at most composed of globules. In this semi-fluid mass cavities (Bläschen, Zellen) are now developed; these, if they remain rounded or polygonal, become the subsequent cells; if they elongate, the vessels.<sup>3</sup>

Schwann's conception is but a slight modification of this idea. Instead of operating in the organ as a whole, the generative activity of the structureless fluid is limited to the confines of individual cells, whereby new cells are formed within preexisting ones. It may well be noted in this connection also that the cellular nature of the chorda dorsalis, cartilage, epithelium, feathers, crystalline lens and adipose tissue, which Schwann described at length in the first part of his contribution, had previously been dem-

<sup>3</sup> Translation by Huxley.

onstrated by Muller, Purkinje, Henle (1838), Hooke (1665), Dutrochet (1824), Raspail (1837) and others. Furthermore, on several occasions Valentin had compared the cellular structure of several of those tissues to that of plants. What we have said previously about Schleiden's lack of originality and accuracy of interpretation may equally well be extended to Schwann. He makes no mention of Dutrochet and Meyen, but by comparing his text with that of Dutrochet, it is difficult to escape the feeling that Schwann was thoroughly familiar with the former's paper. Rich suggests that not only was he familiar with Dutrochet's work but that he may possibly have paraphrased certain passages of it.

As the falsity of Schleiden and Schwann's theory of cell formation was gradually demonstrated, this part of their contribution received less and less notice, and as time went on their fame came to rest primarily on the erroneous belief that they had been the first definitely to announce the doctrine of the independence, individuality and duality of the cell and that it is the elementary unit of organization and structure of all organisms. In the opinion of present-day biologists this stands as the essential part of their contribution. But, as we have emphasized above, it is by no means original. My study of the early biological literature, and I do not regard it as being very complete, leads me to these conclusions. The idea that plants and animals are an aggregate of similar, independent, individual units was first expressed as a philosophical speculation without much factual foundation by Oken in 1805 and 1810. It was restated and confirmed by clear-cut observation for the first time by Dutrochet in 1824, elaborated and extended for plant cells by Meyen in 1828 and 1830, and finally accepted by Schleiden and Schwann in 1838-1839. After the lapse of a century we are better able to judge the relation of Schleiden and Schwann to the history of the cell theory; and we can now realize that, as they themselves thought, their outstanding contribution was the false and wholly misleading attempt to compare cell formation with the formation of a crystal in a mother liquor.

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## PREDECESSORS OF SCHLEIDEN AND SCHWANN

PROFESSOR EDWIN G. CONKLIN

IN science no less than in the material universe it is difficult if not impossible to find the real beginnings of anything, for every event is the result of many preceding ones. In short, there is no creation *de novo* in either the material or the intellectual universe. In an extremely interesting article in the *Scientific Monthly* for December, 1937, entitled "Who Invented It?," S. C. Gilfillan lists the numerous reputed inventors of the telegraph, the friction match, the barometer, the telephone, the airplane, wireless, the steamboat and other modern inventions; and as to the ancient inventors of the wheel, the pulley, the boat, the sail, history is silent: yet in each and all of these inventions we may be sure that there were many cooperators. The fact is that all discovery and all science are social functions. Their progress is possible only by the conscious or unconscious cooperation of many minds.

These remarks apply with especial force to the origin of the cell theory. The present symposium was designed to mark the centenary of the cell theory of Schleiden and Schwann. We are accustomed to celebrate anniversaries of births, decades of science, jubilees of men and institutions, centuries of progress, millennia of world history. We pick out some event of 1838 and celebrate its centenary in 1938, as if it had no antecedents, as if it were a creation rather than an evolution.

The cell theory in its fundamental features is older than either Schleiden or Schwann. Their cell theory was a special and, in important respects, an erroneous one. There is no present biological interest in their theory, and it is amazing that we still continue to call it after them, as if they were its sole inventors, thus embalming the names of real scientists with one of their most serious blunders. It suggests the distinction conferred upon "Bahn" by an

English translation of the German phrase "Bahn-brechende Werke" as "The pioneer work of Bahn."

But mankind desires to concentrate honors on individuals, to pick out persons to love or hate or admire, rather than to deal with multitudes of persons or causes, and so we still speak of the cell theory as if Schleiden and Schwann discovered cells or first proposed that they are the universal units of organic structure and function. However, this is far from the case. As is well known, cells were first seen, named, described and figured by Robert Hooke, an English physician, mathematician and architect, 170 years before the work of Schleiden and Schwann. In 1667 Hooke published his "*Micrographia*," in which he described among many other things the little chambers or cells which he had seen with his microscope in sections of cork. In 1675 and again in 1679 Marcello Malpighi, an Italian anatomist, physiologist and physician, published two folio volumes which justifies his title of "creator of scientific botany." He distinguished parenchyma from fibrous tissue and air tubes from sap vessels. For the elements of the parenchyma he used the term "utriculi." Nehemiah Grew, English botanist and secretary of the Royal Society (1677), published his "*Anatomy of Plants*" in 1682, showing that the parenchyma of plants is composed of vesicles or closed spaces in a homogeneous ground mass.

During the next hundred years, several botanists and anatomists saw and figured the utricles or vesicles in plants and animals. The most notable of these was Caspar Frederick Wolff. His doctoral thesis for the M.D. degree was published in 1759 when he was only 26 years old; it was entitled "*Theoria Generationis*," and it marks an epoch in the study of the development of plants and animals. Wolff showed that in their development the parts of plants are composed of utricles, and "the particles which constitute all animal organs in their earliest inception are little globules which may always be distinguished under a microscope of moderate magnification." V.

Sachs in his "History of Botany" says that this was the most important work of the period between Grew (1682) and Mirbel (1802). "It was Wolff's doctrine of the formation of cellular structures in plants which was in the main adopted by Mirbel." (Sachs.)

For more than 100 years the words utricles, vesicles or globules were used to designate these constituent parts of animals and plants, and only in the beginning of the nineteenth century did Hooke's term "cell" again come into use. In 1808 and 1809 Brisseau de Mirbel, professor of botany in the Musée d'Histoire Naturelle in Paris, published a notable work on his theory of plant organization ("Theorie de l'organisation végétale"). The general conclusions of this work were that "The plant is wholly formed of a continuous cellular membranous tissue." In a set of "Aphorisms" that he had prepared to accompany a large plate illustrating the finer structure of plants he wrote, "Plants are made up of cells, all parts of which are in continuity and form one and the same membranous tissue." It is apparent from this that while Mirbel recognized the universal presence of cells in plants he also regarded them as bound together in a membranous tissue.

Professor John H. Gerould, in an important paper entitled "The Dawn of the Cell Theory" (*Scientific Monthly*, March, 1922), has shown that the great French naturalist, Lamarck, deserves to rank as one of the founders of the cell theory. In his "Philosophie Zoologique," published in 1809, he says: "No body can possess life if its containing parts are not a *cellular tissue*, or formed by *cellular tissue*." Again:

Thus every living body is essentially a *mass of cellular tissue* in which more or less complex fluids move more or less rapidly; so that if this body is very simple, that is without special organs, it appears homogeneous and presents nothing but *cellular tissue* containing fluids which move within it slowly; but if its organization is complex all its organs without exception, as well as their most minute parts, are enveloped in cellular tissue, and even are essentially formed of it.

In the second volume of his great work, "Philosophie Zoologique," Lamarck devotes an entire chapter to cellular tissues, in which he says:

It has been recognized for a long time that the membranes that form the envelopes of the brain, of nerves, of vessels of all kinds, of glands, of viscera, of muscles and their fibers, and even the skin of the body are in general the productions of *cellular tissue*. However, it does not appear that anyone has seen in this multitude of harmonizing facts anything but the facts themselves; and no one, so far as I know, has yet perceived that *cellular tissue* is the general matrix of all organization, and that without this tissue no living body would be able to exist nor could have been formed. Since the year 1796 I have been accustomed to set forth these principles in the first lessons of my course.

Everywhere Lamarck speaks of *cellular tissue*, and apparently neither he nor Mirbel thought of the cell as an independent unit. This idea was more clearly expressed by Dutrochet, a French physiologist and physicist, in 1824 in the following words:

All the organic tissues of animals are actually globular cells of exceeding smallness, which appear to be united only by a simple adhesive force; thus all tissues, all animal organs, are actually only a cellular tissue variously modified. This uniformity of finer structure proves that, organs actually differ among themselves merely in the nature of the substances contained in the vesicular cells, of which they are entirely composed.

Another French naturalist who seems to have escaped recent notice was J. P. F. Turpin, who published in 1826 a remarkable memoir ("Organographie microscopique elementaire et comparée des vegetaux") with a title so complete that it forms an abstract of the contents:

Observations on the origin and first formation of cellular tissue, on the vesicles composing this tissue, considered as distinct individualities having their own vital center of vegetation and propagation and destined to form by agglomeration the composite individuality of all those plants whose organization is composed of more than one vesicle.

In 1830 the German botanist, Meyen, in his "Phytotomie" wrote: "Plant cells appear either singly so that each one forms a single individual, as in some algae and fungi, or they are united together in greater or smaller masses, to constitute a more highly organized plant. Even in this case each cell forms an independent isolated whole; it nourishes itself, builds itself up, and elaborates raw nutrient materials, which it takes up, into very different substances and structures." He even spoke of such cells as "little plants inside larger ones." Meyen also de-

scribed the circulating movement of cell contents, which had previously been observed by Corti in 1774 and by Treviranus in 1811. In his great three-volume work on "Pflanzenphysiologie" (1837), Meyen described cells as the "essential elementary organs of assimilation and construction."

The English botanist, Robert Brown, in 1831 discovered the fact that nuclei are present very generally in plant cells. He called attention to the fact that nuclei had previously been seen and figured in cells by Meyen, Purkinje, Brogniart, Braur, *et al.*, but they had been regarded as unimportant. Brown recognized nuclei as important organs of the cell, and his work marks a major stage in the development of the cell theory, especially in regard to the origin of new cells.

Cell division had been seen in filamentous algae by Turpin in 1826 and by Dumortier in 1832. Hugo von Mohl described the formation of division walls separating daughter cells in 1835, and four years later (1839) he figured and described the division of spore mother cells in the scale moss, *Anthosceros*, some of his figures (Figs. 21-23) suggesting that he had seen mitosis. Meyen in his "Neues Systems der Pflanzen-physiologie" (1838) said that cell division is everywhere easily and plainly seen in *Confervae*, *Mycelia*, *Chara* and also in terminal buds and root tips of *Phanerogams*.

All this significant work on cells preceded the famous publication just one hundred years ago by Mathias Schleiden, professor of botany at Jena, entitled "Beiträge zur Phytogenesis" (1838). There is no doubt that Schleiden was a distinguished botanist and that he contributed much of importance to an understanding of the genesis of plant tissues, but so far from his being the founder of the *cell theory* it can be truly said that his contributions to this great theory were inferior to those of many of his predecessors. It is one of the amazing facts of scientific history that in many biological textbooks Schleiden is called the founder of the cell theory,

as if he had first discovered that all tissues of plants are composed of cells or that the cell is the universal unit of organic function as well as structure. His own particular contribution was in his opinion the discovery of the way in which new cells arise, and yet this has been known for a hundred years to be not only fundamentally wrong but even fantastic. It is still supposed by some biologists that he first set forth the conclusion that the cell leads an independent life. In the beginning of his famous "Contributions" he says:

Each cell leads a double life: an independent one pertaining to its own development alone, and another incidental, in so far as it has become an integral part of a plant. It is, however, apparent that the vital process of the individual cell must form the very first, absolutely indispensable basis of vegetable physiology and comparative physiology.

Yet thirty years earlier Mirbel had expressed this thought, and twelve years before Turpin had stated it with great clarity, while eight years earlier it had been set forth by the famous German botanist, Meyen, in a still clearer and more accurate manner.

So far as the genesis of new cells is concerned, Schleiden's fantastic views that granules (nucleoli) within cells become cytoblasts (nuclei) and that on one side of these a membrane arises like a watch crystal on a watch to form new cells within the old ones—all this could be charitably set down to that liability to error which we call experience, if it were not for the fact that Schleiden is so lacking in charity toward his predecessors, some of whom happened to be right. For example, he says: "Sprengel's pretended primitive cells have long since been shown to be solid granules of *amylum*. To enter upon Raspail's work appears to me incompatible with the dignity of science. Mirbel does not make any allusion to the process of cell formation." Of Meyen's work he says: "I still have many doubts, the solution of which I had hoped to have found in his '*Physiology*,' but hoped in vain." He either underestimated or ignored the work of Mirbel, Meyen, Turpin and especially von Mohl on cell division. On the whole



one gets a very unpleasant picture of Schleiden's relations to his predecessors and contemporaries, and the question forces itself upon us, "How did he come to be recognized as the founder of the cell theory?" I once heard a distinguished physiologist say that there are two ways to gain recognition, either brag or fight. It seems to me that Schleiden did both. But while he was not the founder nor even an important contributor to the cell theory he did make important contributions to the transformation of embryonic cells into the tissue cells of plants. Nevertheless, it is still a mystery how it has happened that he occupies so high a place in the annals of science; his cell theory had no great significance for botany, since it met with immediate and open opposition by all those who had championed cell division as the method of cell genesis, and especially by Meyen (1839), who stoutly maintained that cells arise by self-division.

Julius von Sachs, in his celebrated "History of Botany," says of Schleiden and his work (p. 188):

Endowed with too great love of combat, and armed with a pen regardless of the wounds it inflicted, ready to strike at any moment, and very prone to exaggeration, Schleiden was just the man needed in the state in which botany then was. His first appearance on the scene was greeted with joy by the most eminent among those who afterwards, contributed to the real advance of the science, though their paths it is true diverged considerably at a later period, when the time of reconstruction was come. If we were to estimate Schleiden's merit only by the facts which he discovered, we should scarcely place him above the level of ordinarily good botanists; we should have to reckon up a list of good monographs, numerous refutations of ancient errors and the like; the most important of the theories which he proposed, and over which vigorous war was waged among botanists during many years, have long since been set aside. His true historical importance has been already intimated; his great merit as a botanist is due not to what he did as an original investigator, but to the impulse he gave to investigation, to the aim and object he set up for himself and others, and opposed in its greatness to the petty character of the text-books. He smoothed the way for those who could and would do great service.

Again, after sketching the earlier work on the cell theory, von Sachs says:

Schleiden's behavior was different. After having somewhat hastily observed the free cell formation (*sic*) in the embryo sac of phanerogams in 1838, he proceeded at once to frame a theory upon it which was to apply

to all cases of cell formation, and especially to that in growing organs. The very positive way in which he announced this theory and set aside every objection which was made to it combined with his great reputation at the time, at once procured for it the consideration of botanists generally. (p. 311.)

Schleiden's theory of cell formation arose out of a curious mixing together of obscure observations and preconceived opinions . . . his theory did not rest on any thorough course of observation. (p. 323.)

We make acquaintance with Schleiden's theory of cell-formation in its original form, if we turn to his treatise, "*Beiträge zur Phytogenesis*" (1838). The work begins with some remarks on the general and fundamental laws of human reason, etc., discusses the literature of cell-formation in a few lines without mentioning von Mohl's numerous observation, goes on to mention the general occurrence of the nucleus . . . then occupies itself with gum, sugar and starch, and at last comes to the main subject. (p. 323.)

Then follows his erroneous description of new cell formation and the contradictions which it aroused by Unger, von Mohl and finally Nägeli.

The first result was that Schleiden found himself obliged to accept the cell-division established by Nägeli in algae and the mother cells of pollen as a *second kind of cell-formation*; thus began the movement in retreat which was destined to end in the following year (1846) with the overthrow of Schleiden's theory. (p. 331.)

Theodore Schwann, the distinguished professor of anatomy at Louvain and Liège, took over the erroneous views of Schleiden as to cell genesis and proceeded to apply them to animal cells. Dutrochet (1824), Purkinje (1837) and Valentin (1838) had observed and described animal cells and compared them with plant cells, but only Dutrochet before Schwann had taught that all the many kinds of animal tissues are everywhere derived from cells as the elementary type of organism. Schwann held that all the different kinds of cells are morphologically related because they all arise by the same process, namely, from granules (nucleoli) which become nuclei and which in turn give rise to the cell body. Unlike Schleiden he held that this genesis could take place in spaces between cells, as well as within mother cells. These erroneous views persisted for a long time under the caption of "free cell formation." Fifty years ago I heard this idea presented in lectures on general biology.

The work of Schwann formed the basis of the theory of the "cell state," which maintained that "cells are organisms and that entire animals and plants are aggregates of these organisms arranged according to definite laws." This theory had a long life and is still probably true in part, but in its extreme form its inadequacies were pointed out by Whitman (1893) and by many experimental embryologists, who have called attention to "the organism as a whole."

The principal contributions of both Schleiden and Schwann were in determining the cellular origins of tissues and not, as they supposed, the origins of new cells. They were not the first to develop this tissue theory, but they were important contributors to it. In view of the fact that all discoveries are based upon previous ones and that science is possible only by such cooperation, I suggest that it would be more accurate as well as more becoming to strike out of our literature these personal possession tags attached to important discoveries, such as the foramen of Monro, the islands of Langerhans, or the cell theory of Schleiden and Schwann.

*(This symposium will be concluded in the January-February issue.)*

# MASS MUTATION IN THE FLORIDA STOCK OF *DROSOPHILA MELANOGASTER*<sup>1</sup>

(Details of an old experiment reinterpreted)

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IN 1929 I described<sup>2</sup> a case of what looked like mass mutation after treatment of larvae of Florida stock with heat shocks. Though it has since been confirmed by a number of authors that heat shocks increase the mutation rate beyond the amount of an ordinary temperature function, no similar case of mass mutations has been found. I have myself performed since innumerable heat shock experiments (not published) without finding a similar case, and the same is true for other authors. The majority of geneticists therefore assume tacitly or openly that the original finding was based upon an experimental error, this being the easiest escape from uncomfortable facts. But there was no experimental error, as will be easily seen from an inspection of the details of the experiment. These have not been published thus far, as I was waiting either for a successful repetition or an explanation of the results. This, I think, can now be given: What looked like an effect of the heat treatment was nothing but a chance coincidence of one of the rare cases of spontaneous mass mutation with the heat experiments.

As a matter of fact the correct explanation could have been derived already from certain points in the preliminary description of the facts, which excluded the original explanation. But just as I was blinded by the coincidence with a heat experiment, neither did others who repeated, quoted or criticized the experiment perceive the possibility of spontaneous mass-mutation as the proper explanation. But during the intervening ten years five more cases of mass-mutation, closely paralleling this first one, have been

<sup>1</sup> Assistance rendered by the personnel of Works Progress Administration Official Project No. 465-03-3-192 is acknowledged.

<sup>2</sup> *Biolog. Centralbl.*, 49, 1929.

found and described more or less thoroughly. I myself found two, which, this time, are being analyzed completely, and the very interesting details will be presented in time. (Unfortunately the completion of the work has been since retarded by enforced long interruptions.<sup>3</sup>) One case each was found by Plough and Holthausen<sup>4</sup> and by Demerec,<sup>5</sup> both involving the same Florida stock as my first case. Recently another case, again of the same general type as the others, was found by Valadares<sup>6</sup> and it ought to be added that Spencer<sup>7</sup> had concluded from statistical studies that mutants in *Drosophila* do not appear haphazard, but in clusters. Mass mutation, then, is an apparently rare (?) but absolutely proven phenomenon, which will be of the greatest theoretical importance as soon as we shall know what actually is happening in these cases. Under these circumstances it seems justifiable to publish the details of the first observed case, though a satisfactory analysis was neglected at that time, owing to the wrong interpretation. The material used for this experiment was a long inbred Florida line which did not show any departure from Wild-type in the stocks, in the isolated one-pair bottles which furnished the experimental material or in the controls, derived from the same stock. Unfortunately no controls with brothers and sisters of the experimental flies were run, which would have shown up the situation at once. (Cousins of different grades furnished the controls.) Whatever heterozygosity or contamination could have been present in the material ought, however, to have become visible in time in the actual controls, but for the remote possibility that only the one pair which started the experiment had been contaminated by five different stocks and none of his sisters and brothers who furnished the controls, which were run in huge numbers. We shall see below that one definite finding would have required a con-

<sup>3</sup> See Preliminary Note, *Proc. Nat. Acad. Sci., Washington*, 1937.

<sup>4</sup> *AM. NAT.*, 65, 1937.

<sup>5</sup> *Genetics*, 22, 1937.

<sup>6</sup> *Revista Agronóm.*, 25, 1938.

<sup>7</sup> *AM. NAT.*, 69, 1935.

tamination at least three generations before the experiment started, with an additional, improbable series of favorable events. There is in addition one mutant form which had never existed before in the laboratory (as a matter of fact a new one) and other features to be reported, which exclude absolutely an experimental error. The perfectly parallel features of the results of other authors, just mentioned, add to the safety of this conclusion.

The bottles made up for the heat treatment contained about five pairs of Florida flies, brothers and sisters, all derived from a one-pair mating. There were twelve bottles, containing brothers and sisters, and in addition each group of animals was permitted to lay eggs four times for 24 hours, thus giving 48 bottles, 4 bottles to one group of brothers and sisters each. These were used for the heat exposures of larvae, which kills or sterilizes a majority of individuals, though not in all cases. Therefore only a relatively small number of successful matings could be obtained from the treated flies. This again led to the frequent use of mass matings (see below). The parents of this experiment then were all brothers and sisters, and the flies from heat-treated bottles were all grandchildren of one pair of Florida flies. For the purposes of description the flies from heat-treated larvae may then be called  $F_2$ .

As was described in my paper of 1929 and elaborated in more detail in 1935,<sup>8</sup> the heat-treated flies show in a rather regular manner the phenotypes of many standard mutants in not-heritable form as so-called phenocopies. But in addition to these a small number of individuals occurred in  $F_2$  showing the types which appeared as "mutants" in later generations. These individuals are of special importance. Unfortunately the ratio in which they appeared is not accessible to analysis, because 4 to 5 pairs had to be mated to secure enough offspring after treatment. The lethality of treated larvae in addition might be selective. But we shall see that the ratios in  $F_3$  are not mendelian either, and therefore we may assume that the non-

<sup>8</sup> *Zeitschr. ind. Abstammgl.* 69, 1935.

mendelian ratios in  $F_2$  are also significant. From 20 of the 48  $F_2$ -bottles an  $F_3$  generation could be obtained. Four of these 20 bottles contained flies phenotypically identical with the "mutants" appearing in further generations. The few fertile ones inherited their type, as will be seen. The four  $F_2$  cases are (normal means neither of the latter mutant types; abnormal bristles and inconspicuous eye-colors were not checked in this experiment) :

No. II Ab 70 ♀ ♂ normal 4 ♀ 3 ♂ sooty

No. III Bc 114 ♀ ♂ normal 1 ♀ sooty, aristapedia, rolled

No. IV Ab 57 ♀ ♂ normal 1 ♂ sooty

No. IV Ac (same parents as before) 26 ♀ ♂ normal 1 ♀ 2 ♂ sooty

Of these "mutant" types only some sooty of the first bottle were fertile. The greatest interest attaches to the one female sooty, aristapedia, rolled, coming from a bottle with a large number of surviving offspring. Let us assume that a contamination had occurred in the Florida stock. Both aristapedia and rolled existed in our stock room, but not in combination. An origin of this individual by contamination would have required: (1) Three independent contaminations in the same bottle by  $e^s$ ,  $ss^a$  (aristapedia) and  $rl$  (rolled) flies; (2) recombination of  $ss^a e^s$  (III) and rolled (II); (3) crossing over between  $ss^a$  and  $e^s$  (only 12 units apart), (4) union of two crossover gametes simultaneously containing the recombination with rolled. As all this would have required at least three generations (and an additional series of pleasant coincidences), it is not to be understood why neither P,  $F_1$  or the thousands of flies of the controls ever showed anything. The numerical results of further generations will also exclude the idea of experimental error. It might be added that the bottle in question contained only 30 dead larvae. (These are, of course, the facts which ought to have led to the correct explanation).

From the twenty  $F_2$  broods which contained fertile flies 39  $F_3$  cultures were obtained partly from pair matings, partly from matings of more than one pair. The majority of these  $F_2$  bottles contained a normal number of flies, thus

making a selection of gametes improbable. This fact, by the way, points out that the high degree of sterility in  $F_2$  was at least partly due to genetic causes. Let us first consider the offspring of the  $F_2$  III Bc, which had yielded the sterile aristapedia sooty rolled female, and also the contents of the three other bottles, III B, III Ba, III Bb, which had been stocked with the same parents for 24 hours each. Twelve different  $F_3$  were obtained. From these the following were derived from one pair matings:

(1) Male and female both showing different phenocopic changes of wings, otherwise normal. But it is possible that the female was rolled, as the phenocopy rolled can not be distinguished from the mutant rolled.

$F_3$  (IIIBc<sup>2</sup>d) consisted of:

346	♀	♂	normal
20	♀	♂	sooty
10	♀	♂	rolled
10	♂		white

I add at this point that sooty, rolled, aristapedia, white, have been extracted from this and other broods and turned out to be identical with the standard mutants of that name. (Regarding multiple alleles see below.) The possibility of a position effect at the loci may be excluded by the perfectly normal breeding of the extracted recessives.

In this brood the white might have been produced by a mutation in the mother's ovogonia. But the autosomal recessives, sooty and rolled, could have been produced only in mendelian ratios if a former simple mutation were involved and the parents had been heterozygous.

(2) ♀ ♂ with changed wings, otherwise normal.

No. III B<sup>2</sup>a 87 ♀ ♂ 1 ♂ aristapedia

Again not explainable on the basis of a former mutation or contamination.

(3) ♀ ♂ as before.

No. III Ba<sup>2</sup>d. 24 ♀ ♂ normal.

The other 9  $F_3$  broods were obtained from 2 to 5 pairs of  $F_2$  flies. As a very large number of treated  $F_2$  flies were



sterile in these experiments, this method of breeding had to be used, as the experiment was meant to discover mutants. Actually of 52 more than one pair-matings 32 produced offspring; of 50 one pair-matings only 7 were fertile. It is to be assumed therefore that among the matings with 2 or more pairs, up to 5, frequently the offspring will actually be the offspring of one pair, though one can never be sure. Five of the 9 successful matings gave only normal offspring. Two of these were bred to  $F_4$ , of which again one bred true to wild type. The other threw in  $F_4$  a few sooty and aristapedia individuals among a multitude of normals (number not recorded but not relevant because more than one pair of parents was used). The other 4 successful  $F_2$  matings were (all parents normal but for phenocopic wing characters):

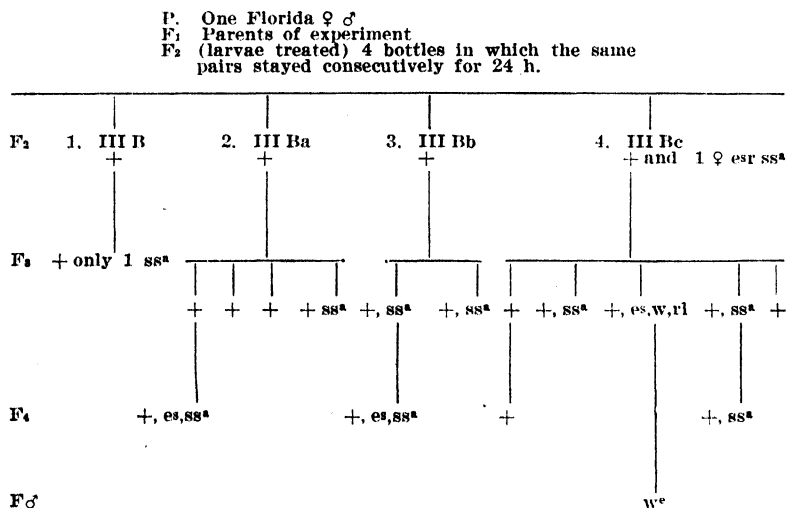
No. III Ba <sup>2</sup> b	24 ♀ ♂ normal	13 ♀ ♂ aristapedia	1 ♀ giant
III Bb <sup>2</sup> b	(2 pairs) 240 ♀ ♂ normal	24 ♀ ♂ aristapedia	
III Bb <sup>2</sup> c	88 ♀ ♂ normal	24 ♀ ♂ aristapedia	
III Bc <sup>2</sup> a	116 ♀ ♂ normal	4 ♀ ♂ aristapedia	
III Bc <sup>2</sup> g	220 ♀ ♂ normal	11 ♀ ♂ aristapedia	

Many different  $F_4$  were bred from these. Normal flies gave either normals, or normals and aristapedia, or normals, aristapedia, sooty and aristapedia-sooty. Also aristapedia ♀ ♂ threw sooty in  $F_4$ . As most of these broods—with the exception of those in which the mutant types were extracted—were made from more than one normal pair, numbers are not important. But in breeding from single, not mutant, pairs again non-mendelian ratios were observed. Thus the giant ♀ from III Ba<sup>2</sup>b bred to a normal brother yielded hundreds of normal flies and only a few individuals (number not recorded) aristapedia. In  $F_5$  a few giants were recovered and a stock could be established, which later was lost.

We return now to the normal  $F_2$  pair, which in  $F_3$  threw sooty, rolled and white, but no aristapedia, present in many other  $F_3$  of the group. Neither did further generations contain aristapedia, and nothing special happened except for the white locus. The  $F_3$  white ♂ were crossed to normal sisters and recovered according to expectation in half of

the  $F_5$  males. But a few of these were no longer white but eosin. Tested with standard eosin and bred over attached —X they turned out to be real eosin males. When later a homozygous white stock was established it threw besides eosin a few individuals with different eye-colors, which my color vision did not permit me to analyze; however, they appeared to Dr. C. Stern, who worked at that time with me, to be identical with other white alleles. Unfortunately the analysis was not continued, as the work then was going in a different direction. Also the sooty flies produced in these experiments turned out to be of different degrees. By selection, at least three different grades of sooty-ebony were established and bred for some time as different alleles of  $e'$ . But no further analysis was made.<sup>9</sup> The following pedigree illustrates this first group of facts:

PEDIGREE No. 1



Let us turn now to the second case in which the treated  $F_2$  flies were not all normal. The bottle IIab, derived from

<sup>9</sup> In fact the work was interrupted at that time, as I was leaving for the Far East in the interest of my work on geographic variation. At that time I thought that the experiment might be easily repeated later with similar results, assuming erroneously that the heat shocks were involved. The mutants which appeared were not considered worth keeping as they were obviously identical with the standard ones.

the same grandparents but different parents as the former series, had contained after the heat treatment 70 normal flies, 6 ♀ 1 ♂ sooty. The grandparents had not been sooty; a contamination of the parents with recessive sooty could not have become visible in this generation. The numbers of the following generation will not agree with such an explanation either. (A contamination of the heated bottle itself is excluded by the complex individual discussed before and by the fact that all mutant types have appeared in some  $F_3$  from normal  $F_2$ . A contamination involving mating with the  $F_2$  parents could not produce the  $F_3$  types. In addition the three ebony alleles which were later extracted had never been in our stocks. Only one successful pair mating was obtained, namely, from a sooty pair. This produced 231 ♀ ♂ sooty, 1 ♀ kidney (not sooty!), 1 ♀ aristapedia (11Ab<sup>2</sup>b) (not sooty), 8 ♀ ♂ sooty and kidney. The pair then was homozygous for ebony. But it threw in addition one ♀ each of kidney and aristapedia (not sooty) and 8 kidney ebony combinations. Notice the ratios of the new types which exclude any idea of an error. Here kidney flies were found, missing in the first described line. They were extracted and bred, and as they represented a new type were continued when the other lines were discarded. Only recently two of my former students have analyzed this line (Gottschewsky and Ma, 1937)<sup>10</sup> and they found that it contained two new kidney alleles. (This paper contains the erroneous statement that the mutant is derived from vg-stock. It came from pure Florida stock. The error is based upon an oral communication that kidney appeared later rather frequently in pure vg-stocks.)

Another  $F_3$  was obtained from two pairs, the females sooty, the males normal (II Ab<sup>2</sup>c). The offspring consisted of 303 ♀ ♂ normal, 2 ♀ 1 ♂ aristapedia. Again aristapedia appeared in non-mendelian numbers. Two more broods from more than one normal pair were obtained.

(1) II Ab<sup>2</sup> ♀ ♂ normal, but a little dusky (probably sooty heterozygous) 285 ♀ ♂, 1 dwarf, some individuals dusky.

(2) II Ab<sup>2</sup>d 184 ♀ ♂ +, 9 ♀ 5 ♂ light sooty, 1 ♂ light sooty and aristapedia, 1 ♂ dwarf.

<sup>10</sup> *Zeitschr. ind. Abstammgl.* 72.

Out of the  $F_4$  derived from these  $F_3$  the following may be recorded:

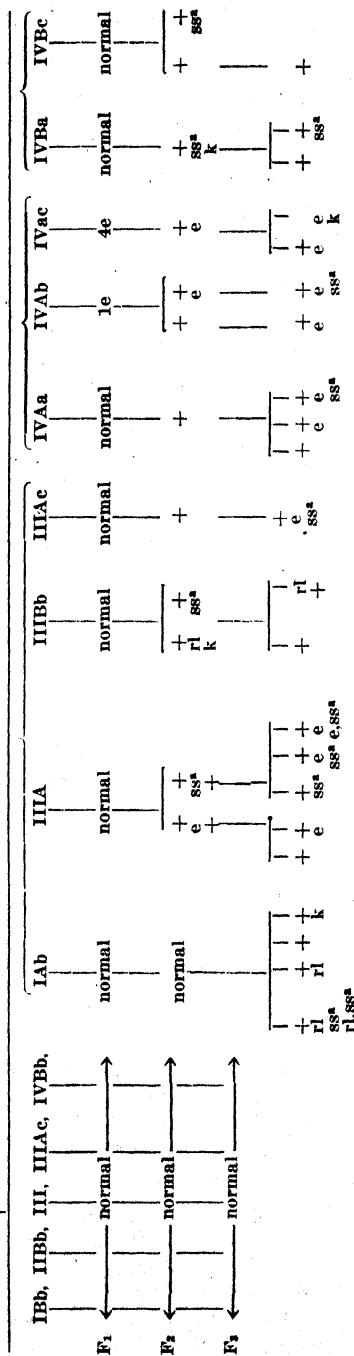
- (1) from II Ab<sup>2</sup>b (parents one pair sooty)  $F_3$  was all sooty plus a few kidney, aristapedia.
  - II Ab<sup>2</sup>b<sup>2</sup>a ♀ aristapedia × ♂ sooty  
sooty, sooty-kidney and kidney. Only 3 kidney individuals not sooty. Sooty: sooty-kidney = 104: 31.
  - II Ab<sup>2</sup>b<sup>2</sup>c ♀ kidney × ♂ sooty  
118 ♀ ♂ sooty, 16 kidney-sooty, 4 ♀ 6 ♂ kidney, 1 ♀ +
  - II Ab<sup>2</sup>b<sup>2</sup>g ♀ sooty × ♂ sooty-kidney  
52 ♀ ♂ sooty, 24 ♀ ♂ kidney
  - II Ab<sup>2</sup>b<sup>2</sup>b ♀ ♂ sooty, more than one pair  
63 sooty, 7 sooty kidney, 3 aristapedia.
  - II Ab<sup>2</sup>b<sup>2</sup>d dto  
sooty and sooty-kidney
  - II Ab<sup>2</sup>b<sup>2</sup>d dto  
sooty and sooty-kidney
- (2) from II Ab<sup>2</sup>c derived from two pairs sooty × normal  
 $F_3$  was all normal but for a few aristapedia  
 $F_4$  from normal parents contained normals, sooty and aristapedia in one case, only normals in the other.
- (3) from II Ab<sup>2</sup>d, containing only normals, eight sooty and one aristapedia:
  - II Ab<sup>2</sup>dd ♀ light sooty × ♂ no  
all normal or light sooty or sooty (not classified).
  - II Ab<sup>2</sup>dg ♀ ♂ light sooty  
116 ♀ ♂ sooty, 17 ♀ ♂ sooty kidney, again a non-mendelian ratio for the first appearance of kidney.
- (4) from II Ab<sup>2</sup> which contained only normals.
  - 2  $F_4$  all normal
  - 1  $F_4$  135 normals, 9 aristapedia

These data may suffice to show (1) that sooty was homozygous in those  $F_2$  flies which had shown it. (2) that in the offspring of this line again aristapedia and also kidney appeared in a rather erratic fashion, certainly not in mendelian ratios, whenever pair matings were made; (3) that rolled and white were absent in this line derived from brothers-sisters of the foregoing, whereas kidney was absent in the former one.

It is hardly necessary to point out all the details of the other lines, derived from other brothers and sisters in this experiment. I may summarize their parallel results in the following way:

PEDIGREE No. 2

♀ × ♂ Florida



(1) Five groups, containing in the treated offspring none of the mutant types, remained normal in  $F_3$  and  $F_4$ .

(2) Three such groups produced only normal offspring in  $F_3$ , but in  $F_4$  two threw sooty and aristapedia and the third aristapedia, rolled and kidney.

(3) All the others behaved essentially like the groups described in detail, namely:

(a) One group threw already a few sooty individuals in  $F_2$ .  $F_3$  from normals contained mostly normals and a few sooty, in  $F_4$  in addition a few aristapedia and kidney appeared.

(b) The others were normal in  $F_2$ . In  $F_3$  one series threw only aristapedia and a single kidney individual among hundreds. The other series (3 bottles from same parents) produced in  $F_3$  sooty, aristapedia, rolled and kidney in significant numbers.

A generalized pedigree (No. 2) of these last groups is found below. The only details which ought to be recorded are the numbers in which the mutant types appeared.

The following table summarizes the important cases:

No.	+	es	ss <sup>a</sup>	k	rl	Gen.	Mating	Pairs P
I Ab <sup>2</sup> b <sup>2</sup> .....	140	..	..	3	..	F <sub>4</sub>	+++	1
I Ab <sup>2</sup> b <sup>2</sup> 2b .....	ca. 200	..	1	..	..	F <sub>5</sub>	+++	more
I Ab <sup>2</sup> b <sup>2</sup> 2a .....	85	5	..	..	..	F <sub>5</sub>	+++	"
III Aa <sup>2</sup> a .....	159	..	3	..	..	F <sub>4</sub>	+++	3 ♀ 1 ♂
III Aa <sup>2</sup> a <sup>2</sup> a .....	79	..	2	..	..	F <sub>5</sub>	+++	more
III Aa <sup>2</sup> a .....	103	1	3	..	..	F <sub>5</sub>	+++	"
III Aa <sup>2</sup> a <sup>2</sup> b .....	72	..	3	..	..	F <sub>5</sub>	+++	"
III Ab <sup>2</sup> b .....	448	..	..	1 k, rl	16	F <sub>4</sub>	+++	"
III Ab <sup>2</sup> b <sup>2</sup> c .....	48	..	..	..	1	F <sub>5</sub>	+++	"
III Ab <sup>2</sup> b <sup>2</sup> b .....	39	..	..	..	3	F <sub>5</sub>	+++	"
IV Ab <sup>2</sup> .....	179	11	..	..	..	F <sub>4</sub>	+++	"
IV Ab <sup>2</sup> .....	116	2	..	..	..	F <sub>5</sub>	+++	"
IV Ab <sup>2</sup> f <sub>2</sub> .....	126	3	9	..	..	F <sub>5</sub>	+++	2
IV Ba <sup>2</sup> b .....	281	..	28	1	..	F <sub>4</sub>	+++	more

Among these there is one pair mating, one mating with one male and one with two pairs. A few individuals, sooty, kidney and aristapedia, appeared. In the other cases, up to four pairs were mated. In three or four instances, one of the mutant types was found in such numbers that a mendelian segregation involving one pair of flies is suspected. In the other cases this is highly improbable. If standing alone the broods from more than one pair would of course not be of any value. Taken together with the identical results of one pair matings, they deserve at least to be recorded. It is needless to say that in all these cases we are dealing with the first appearance of the same mutants in the line. Once present, they breed

according to expectation for ordinary recessive mutants, as was always tested for a few generations.

It is regrettable that at the time when these observations were made their real meaning could not be suspected. Under the impression that a case of heat effect upon mutation, including the so-called parallel induction, was being studied, I was only interested in the appearance of hereditary or not-hereditary types and bred accordingly. Thus the chances for a closer analysis were lost which, however, will soon be finished in another case. Nevertheless the material as it stands seems to parallel the other cases so thoroughly that I feel justified in assuming that here a spontaneous process of mass mutation was actually involved.

#### DISCUSSION

A detailed discussion of this case and an explanation of what has happened will be presented together with the full analysis of the more recent cases. In a general way my opinion is already expressed in the preliminary communication, and all work done since shows it to be correct. This opinion is that mass mutation is the result of very small chromatin-rearrangements (so-called gene mutations), which are caused by already pre-existing chromatin rearrangements probably by favoring "illegitimate" (Darlington, Muller) crossing over. As a matter of fact it could be shown that all stocks, which thus far have produced mass mutation, contained one or more rearrangements. This is true for my two recent cases and for Valadares' case. As it has been shown recently (see Goldschmidt, Gardner and Kodani, *Proc. Nat. Acad. Sci., Washington*, 1939) the standard Florida stock contains a large inversion in the third chromosome. In this stock three of the cases have occurred. We might, therefore, summarize the two other Florida cases shortly.

There is first the case published by Plough and Holt-hausen (1937).<sup>11</sup> They crossed wild-type Florida with black-purple-curved males and backcrossed  $F_1$  with the

<sup>11</sup> AM. NAT., 65, 1937.

latter. Among 300 flies there were 7 showing the mutation blistered wing. Three blistered females were mated to normal brothers, and the offspring was inbred for six generations. In each generation a number of not heritable variants were found as well as sterile types. This is, by the way, also the case in my work, and it is exactly what is expected from chromatin rearrangements. In addition a series of mutants appeared, most of them appearing several times. Among these is an extreme plexus, abnormal abdomen and scute. The authors conclude that the "mutating period" was initiated by a genetic change within a single individual, and they think of a mutability stimulating factor as described by Demerec for the Florida stock (see below). No exact pedigree has been published, but as far as the information goes this case closely parallels the one described here.

In a series of papers<sup>12</sup> Demerec reported upon a gene for mutability in Florida stock. In the presence of both second chromosomes from this stock the rate of lethal mutations at many points of the X-chromosome was very high. In addition after crossing this line a high percentage of visible mutants was produced, among them yellow not less than twenty-four times, and others also repeatedly. The lethals found could not be associated with chromatin rearrangements. Demerec does not seem to take any offence at assuming as explanation a mutated gene which caused one and the same locus in another chromosome, and different ones at that, to mutate constantly. This most wonderful of all "genes" ever discovered was unfortunately lost. Homozygous lethal chromatin rearrangements without visible effect are, if not suspected, easily lost.

<sup>12</sup> *Genetics*, 22, 1937.



## SHORTER ARTICLES AND DISCUSSION

### THE CHROMOSOMAL CREPUSCULUM

THERE is no phase of cytological research which is more involved in confusion both of thought and consequently of terminology than is the reduction in number of the chromosomes, which has long been known in connection with reproduction in both plants and animals. This obscurity is the natural result of difficulties both in matters of technique and in observation. Until comparatively recently little has been known of the internal organization of chromosomes. As a result of improvements in methods of fixation it is now clear, in all cases where observations have been made on material in which the size of the chromosomes is sufficient to permit reliable inferences as to structure, that the organization of somatic and reproductive chromosomes is identical. It has been supposed that at the metaphase of division the somatic chromosomes carry only two interwound spirals or chromatids. In contrast the reproductive meiotic chromosomes contain four chromatids. Observations on the plant side in the case of *Trillium*, *Lilium*, *Gasteria* and *Tradescantia* make it clear that as regards internal organization all chromosomes have the same structure at comparable stages of nuclear division. Doubtless were the chromosomes of animals in general as large as those of plants a similar conclusion would and should be reached.

The greatest confusion has obtained in the case of the all-important stage in which the reduction of number in chromosomes occurs in the passage from the somatic to the reproductive cycle. The older view, which for example is presented in the second edition of Wilson's classic, "*The Cell in Development and Inheritance*," is expressed by the following quotation: "*The first indication of numerical reduction appears through the segmentation of the spireme-thread or the resolution of the nuclear reticulum, into a number of masses one half that of the somatic chromosomes. In nearly all higher animals this process first takes place two cell-generations before the formation of the definitive germ-cells.*" Equally categorical statements have been made on the botanical side by Strasburger and others. More recently and notably in the much enlarged third edition of Wilson's classic volume, a very different point of view has been adopted ("*The Cell in Development and Inheritance*," Macmillan

Company, New York, 1928). According to the later view the nuclear filament (spireme) is no longer regarded in general as primitively continuous but constituted by separate chromosomes. In the soma or body these are  $2N$  or  $2X$  in number. In the transition to the reproductive phase somatic chromosomes by the so-called process of synapsis or syndesis become associated in pairs, the supposed synaptic or syndetic mates. In these pairs one element is in general regarded as of female paternal origin and the other of male. Shortly after association, the mates again separate at the anaphase of the first meiotic division. This hypothesis presents a serious logical defect, since it necessarily assumes that the chromosomes of the parent sexes remain permanently distinct, and as a result there can, on a chromosomal basis at any rate, be no crossing over of parental characters such as is quite generally observed genetically in the offspring. It further presents the fundamental difficulty that in reproduction the chromosomes only meet for an obviously futile parting.

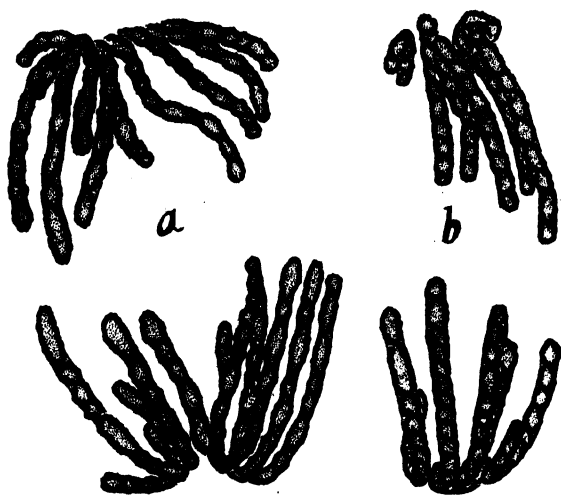
This difficulty has doubtless favored the appearance of the chiasmatype hypothesis of Janssens ("La Cellule," 1909). This theory, criticized by Wilson in his third edition, has been nevertheless adopted by T. H. Morgan and his numerous followers in connection with experimental genetics. In its latest form, the hypothesis assumes that the reproductive chromosomes are not only haploidal in number but also in contrast to the somatic chromosomes in their simplest condition present a single chromatid instead of the two which appear in the diploidal somatic cells. In a manner which remains obscure in the works of these writers the two chromatids of the somatic or sporophytic cells become single as a preliminary to the supposed synaptic pairing. This originally single chromatidal equipment of the reproductive elements is present not only in the first meiotic division but also in the second and is consequently supposed to be characteristic of the gametes or ultimate reproductive elements. As a result of sexual union the chromosomes once more become diploidal in number and also now carry two chromatids, in contrast to the supposed single chromatids of the chromosomes of the reproductive sequence. By some curious logical legerdemain, the somatic chromosomes double in number and carrying two chromatids are supposed to emerge from the resting stage preliminary to meiosis still in the diploid number but now possessed of only single chromatids. In the prophases of the first meiotic division these

single chromatids are imagined to pair and to undergo chiasmatic involvements before separating once more in the second meiotic maturation reproductive division. In the sexual union following meiosis double chromatids are again established in the divisions of the chromosomes of the fertilized egg and the soma of which it is the initial stage.

It is obviously necessary in connection with the involved and too largely hypothetical course of events outlined in the preceding paragraph that the gametes must always possess chromosomes with a single chromatid, and that this assumption must also be made for the two meiotic divisions which lead to their formation. In the case of the higher animals it is quite difficult to arrive at clarity in the situation on account of the relatively small size of the chromosomes and of the close proximity of the appearance of the gametes to the actual meiotic divisions. The higher plants present a very different and much more favorable situation. Here meiosis does not lead directly to the formation of gametes, but these make their appearance in connection with sex-organs or cells formed on a special sexual soma. Thus the confused situation, to which the name *crepusculum* has been applied in the title, is clarified since we have to do with a more or less prolonged series of divisions in cells of the reproductive category. Further, the divisions do not start from an obscure resting phase, extremely difficult to decipher microscopically, but are continuous and consecutive.

Among the higher plants certain of the Monocotyledons have shown themselves as particularly favorable for cytological investigation on account of the large size of their chromosomes. The genus *Trillium* of American and Asiatic distribution has proved to be outstanding in this respect and has been investigated cytologically by a large number of cytologists. They have, however, without exception failed to realize the importance of the gametophytic divisions in connection with any durable hypothesis of the relation of chromosomes to heredity.

It will be useful in the present connection to indicate the organization of the chromosomes in their simplest form as illustrated by the anaphase (the telophase and early prophase present comparable conditions) of nuclear division as exemplified by the soma. Fig. A shows a later anaphase from the root of *Trillium*. Obviously the two groups which foreshadow the formation of daughter cells are characterized by the presence of chromosomes



FIGS. A and B.

in which there are present two reversely coiled chromatids. This situation has been found to exist in a large number of cases, not only in numerous species of *Trillium* but also in *Lilium*, *Erythronium*, *Tradescantia*, *Gasteria*, etc.

In Fig. B is shown the anaphase of the second nuclear division in the sexual soma or gametophyte of *Trillium erectum*. In accordance with the chiasmatype hypothesis of crossing over, the chromosomes of the gametophytic divisions of *Trillium* should contain only a single chromatid. Quite clearly they do not differ in any way from those of the ordinary vegetative soma. We have studied all the divisions involved in the reproductive processes in *Trillium*, including the meiotic as well as the gametophytic ones, and in all instances we have found the chromosomes to present the same organization as the ordinary somatic ones. Similar conditions are found in the male gametophytes, but naturally less clearly as a result of the relative degeneracy of the male sexual generation. Corresponding results have been obtained in the case of genera *Lilium*, *Erythronium*, *Tradescantia* and *Gasteria*.

A detailed comparative examination of the structure of chromosomes in the somatic, meiotic and gametophytic divisions in the present connection have made it clear that their organization as revealed by the microscope is always essentially the same. They contain in every instance two spirally wound chromonemata or chromatids presenting the features of association which in the

case of the meiotic chromosomes has been interpreted as chiasmotypy. If chiasmotypy is present in the chromosomes involved in the reduction division, it is likewise clearly present in all chromosomes, whether reproductive, vegetative somatic or sexual somatic. Since the conditions supposed to be present in meiotic chiasmotypy can not by any stretch of scientific imagination be realized in the divisions of the body cells in animals, or in the sporophyte in plants or in the gametophyte in plants, its significant relation to the meiotic processes and to crossing over is subject to serious doubt. It further requires an organization of reproductive chromosomes fundamentally different from that observed microscopically in other chromosomes, namely, the presence of a single internal chromatid instead of the two chromatids found in all other cases. In the case of the sexual soma or gametophyte of the higher plants it has been shown in the present connection, beyond possible question, that the organization of reproductive chromosomes is precisely the same as in those of the body or soma.

The doctrine of chiasmata, invented to mitigate the logical absurdity of chromosomata meeting at meiosis to part again completely at a later stage, is apparently without adequate foundation, when subjected to the test of a wider critical examination. The relatively new discipline of cyto-genetics presents a luxuriant growth of ephemeral hypotheses, which a ripper and wider knowledge will doubtless prune away. Among the first of these excrescences to be excised is apparently the hypothesis of chiasmotypy, since it is founded on no secure, accurate or extensive basis of cytological facts.

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### THE COLORATION AND COLOR CHANGES OF THE GULF-WEED SHRIMP, *LATREUTES FUCORUM*<sup>1</sup>

THERE are few faunas more complexly and colorfully adapted to their habitat than is the fauna of the floating sargassum weed of the Gulf Stream. One of the foremost among the crustaceans in this regard is the little shrimp, *Latreutes fucorum*, which has an almost endless variety of tints and color patterns, rivalling

<sup>1</sup> Aided by a grant from the J. F. Porter fund, Harvard University.

in diversity those of the Gulf-weed crab, *Planes minutus*, as described by Crozier (1918). Some of the *Latreutes* are uniformly colored pale yellow, yellowish-green, greenish-brown, brown or red and reproduce almost precisely the diverse colors of the algae to which they cling quite tenaciously. Others are mottled, striped or barred and correspond in pattern to the more irregularly colored bits of weed. Some are black or black with white spots and bars, thus resembling to a remarkable extent the purple-black lifeless tips of the algae, together with the encrusting bryozoans. Another and somewhat common coloring is produced by bright blue patches upon the dorsal and lateral surfaces of the animal. In short, it appears that in such crustaceans as *Latreutes* we have one of the highest points of chromatic evolution found in the group. It becomes interesting to us, therefore, to learn something more about the pigments and pigmentary responses of this highly specialized form.

Four kinds of pigments are found in this animal. They are reflecting white, red, yellow and blue pigments. A similar combination of pigments has been described for the chromatophore complex of *Hippolyte varians*, *Leander* and *Palaemonetes* by various investigators. But unlike the littoral forms just described, *Latreutes* has a great abundance of reflecting white pigment which may vary in hue from a yellowish-white throughout the greater part of the integument of the animal to a clear white in patches on the dorsal and lateral surfaces of some color forms. This production of an excessive amount of reflecting yellowish-white pigment can perhaps later be explained in terms of the action of the intense illumination to which these creatures are subjected while floating in weed at the surface of the ocean under a bright sub-tropical sun, because the other two common shrimp of this habitat, *Leander tenuicornis* and *Hippolyte acuminata*, have likewise a very large amount of this pigment. Thus, these animals are all semi-opaque in contrast to the more familiar transparent shrimp.

The relative abundance of the red, yellow and blue pigments, on the other hand, is subject to much more individual variation. From a single small floating mass of weed it was possible to find an animal of any shade ranging from a pale yellow to black. The former would contain almost no blue and red pigments, while the latter would possess a great deal of these.

Fundamentally, the migrational responses of the pigments within the chromatophores of *Latreutes* was similar to that de-

scribed for *Palaemonetes* by Perkins (1928) and Brown (1935). The red and yellow pigments responded to a white background by concentration into the chromatophore centers, and to a black background by dispersion into the chromatophore branches. Darkness produced a concentration of these pigments, as did also an injection of sea-water extract of the eyestalks of *Leander affinis*.

A variation in the pigmentary behavior was seen, however, in the responses of the reflecting white chromatophores in animals kept upon a black background. Darkness or a black background with low intensity of incident light, such as is found in an ordinary laboratory, would call forth a concentration of this pigment. The direct light of a bright sky or sunlight would produce a dispersion of this pigment in spite of the black background. It will require more experiments to determine whether this is an instance of the direct effect of light upon the chromatophore as believed by Stephenson (1934) to hold true for *Leander*, or whether the eye is involved here as it is in background adaptation. At all events, if the theory of Keeble and Gamble (1904) that a dispersed pigment increases in amount and a concentrated one decreases is true, we have a part explanation of the abundance of this reflecting yellowish-white pigment within these animals accustomed to the intense light found in the ocean surface.

An examination of the blue patches which occurred in many of the animals showed them to consist of chromatophores which were apparently filled with a blue pigment and which physiologically behaved as the reflecting white. When a piece of the integument containing a few of these blue chromatophores was treated with 70 per cent. alcohol while being viewed microscopically by reflected light, it was seen that as the alcohol reached the chromatophore the blue pigment was first transformed into red and then dissolved out, leaving a clear white chromatophore which resembled the normal white ones in appearance. This observation, together with the physiological responses of these blue chromatophores, strongly suggest that here it is merely a case of accumulation of the blue pigment within particular white chromatophores and thus masking them.

In view of the recent advances in the knowledge of the physiology of color change in decapod crustaceans it is improbable that the different color patterns are solely the results of the responses of the animals to individual situations, though this may hold true to some extent. Examination of many animals seems to indicate

that here we are dealing with a specific and inborn basic pattern which in an individual animal is repressed or encouraged by light intensity and color of the background. For instance, when clear white patches are present upon the animal they are restricted to particular regions of the body. Comparable white areas quite constant in position were also described by Crozier to be present in *Planes minutus*.

The shrimps vary in the degree to which these white regions are encroached upon or replaced by the colored pigments, a cardiac bar being the one most persistently present. A reddish brown animal having a very prominent white cardiac bar was placed in a black dish in a dimly lighted room. The white pigment of the bar concentrated into chromatophore centers and remained so during the major part of a five-day interval. At the end of this time the animal was subjected again to bright illumination to produce dispersion of the white pigment, but now the area was tinted by red and blue pigments which had formed in this region during the experimental period. And too, the area covered by the whitish patch seemed to have decreased.

Similarly, the blue spots which are upon some animals are probably subject to the same explanation. When found in animals they are more or less constant in magnitude and position.

What is perhaps most interesting in this report is a description of the wide variety of colors and color patterns in an animal which conforms in most simple responses to crustaceans having far less color change ability. *Latreutes* rivals or surpasses in its color varieties *Hippolyte varians* investigated early in this century by Gamble and Keeble (1900). Some aspects of the color changes in this latter species have been recently reinvestigated by Kleinholz and Welsh (1937). Both *Latreutes* and *Hippolyte* should be re-examined in detail in the light of our present knowledge of hormonal control of the chromatophore complex.

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## A NEW ALLELE IN THE WHITE SERIES OF DROSOPHILA AND ITS RELATIONSHIP TO SOME OTHER WHITE ALLELES

THERE are many series of multiple alleles which can be arranged according to phenotype in a simple quantitative series. Their compounds with each other also fit into a sequence in which the same order is observed.

This typical behavior is often ascribed to the familiar alleles of white in *Drosophila melanogaster* of which at least fourteen are now known. Dunn (1935) gives a summary of their colors and interrelations. A new member of this series, pearl ( $w^p$ ), has been reported recently by Steinberg (1937). The mutant appeared on February 17, 1937, as a single male in a stock *fj px sp* (Columbia stock 36) and was extracted from a cross of the original male by Oregon wild type. The new mutant has now been studied in compound with several other alleles of white, and a departure observed from the usual rule that the alleles at this locus fall into the same serial order of effect in both homozygotes and compounds.

Seven alleles of white (eosin  $w^e$ , cherry  $w^{ch}$ , apricot  $w^a$ , honey  $w^h$ , tinged  $w^t$ , pearl  $w^p$ , and white  $w$ ) were employed in this experiment. They are arranged below in order of decreasing darkness:<sup>1</sup>

$$w^e \text{ } \varnothing > w^{ch} \text{ } \varnothing > w^a \text{ } \delta \cong w^{ch} \text{ } \delta \cong w^e \text{ } \delta > w^a \text{ } \varnothing > > \\ w^h \text{ } \varnothing > w^h \text{ } \delta > > w^t \text{ } \delta = w^t \text{ } \varnothing > w^p \text{ } \delta = w^p \text{ } \varnothing > w \text{ } \delta = w \text{ } \varnothing$$

The above sequence follows previous results closely with the exception of  $w^{ch} \text{ } \delta > > w^a \text{ } \delta$  as reported by Dunn. It has been found that  $w^a \text{ } \delta \cong w^{ch} \text{ } \delta$  is probably the condition in the stocks used in the present experiments.

The qualitative contributions made by the alleles white, pearl and tinged have been carefully studied since their color intensities

<sup>1</sup> Definitions of symbols used:  $\cong$  approximately of same intensity, neither sharply nor consistently darker or lighter than;  $>$  definitely and regularly darker than;  $>>$  regularly very much darker than;  $\geq$  definitely darker than in at least 75 per cent. of the comparisons, the rest being borderline cases.

are very similar. However, they may be separated from each other *easily* and *quantitatively*. Thus white and pearl can be told apart with the naked eye. The eye color of the white mutant approximates the flat white of milk, while pearl is aptly described by the light yellow of cream or butter. Age offers no obstacle to separation, for the eyes of pearl flies not over ten minutes old are unmistakably darker than the eyes of white flies which have been aged for a few days. Unlike white, pearl eyes do change slowly with age when their early dull ashen-yellow cast deepens slightly.

The separation of pearl from tinged is not as clear as that of pearl from white, because the color difference between pearl and tinged is more one of quality than of brightness or darkness. Actually pearl appears slightly "darker" or duller than tinged, but tinged is placed closer to the dark end of the series since it shows more of the "red" ingredient. One can see traces of pink in tinged that are never recognized in pearl or in white. Here it should be recalled that the wild-type eye contains yellow, orange and red pigment granules and that the red granules represent the highest state of reduction (Schultz, 1935). It is therefore probable that the presence of pink in tinged indicates the attainment of a higher threshold or state of reduction than the yellow threshold of pearl, but this is not definitely known. The apparent color disparity might just as well be caused by a differential distribution of variously colored pigment granules in tinged and in pearl. However, the threshold distinction is important for the possible bearing it has on the contribution of the two alleles to compounds of each with some third darker allele.

In white, pearl and tinged mutants, males and females have practically identical eye-color intensities. This only shows, qualitatively at least, that the eye color remains constant whether one or two of these sex-linked alleles may be present. However, one has the advantage of knowing from this that in the production of a compound of one of these alleles with some fourth darker allele, the addition of the gene of the darker allele, rather than the addition of the X chromosome in which it rests, will probably be the more material factor in influencing the phenotype of that compound.

Having established the color differences among the pure types, and having clearly shown the relation of pearl to white and tinged, compounds were made up in which the effects of honey, tinged, pearl and white could be compared in compound with the

darker alleles, eosin, cherry and apricot. These were obtained by crossing stocks containing the desired alleles; the stocks used were not known to be otherwise isogenic. When hatching began, the culture bottles were cleared approximately every hour, so that the eye colors of the female compounds could be compared at early and known ages. A comparison of older females of known age was made by aging the newly hatched females in separate vials. The eyes were examined in blue-green light reflected from a white porcelain plate. An effort was made to eliminate shadows, bright areas and chromatic aberration. Frequently the positions of the flies on the porcelain were interchanged to determine whether the observed differences were actual or due merely to variations in intensity of illumination of the field.

The results may be summarized by arranging the compounds in order of decreasing darkness from left to right.

Apricot compounds:

average age 1.5 hours  $w^h/w^a >> w^t/w^a > w/w^a = w^p/w^a$

average age 5 hours  $w^h/w^a >> w^t/w^a > w/w^a \cong w^p/w^a$

Cherry compounds:

average age 1 hour  $w^h/w^{ch} >> w^t/w^{ch} > w/w^{ch} \cong w^p/w^{ch}$

average age 8 hours  $w^h/w^{ch} > w^t/w^{ch} \cong w/w^{ch} > w^p/w^{ch}$

Eosin compounds:

average age 2.5 hours  $w^h/w^e >> w^t/w^e \cong w/w^e > w^p/w^e$

average age 5 hours  $w^h/w^e > w^t/w^e = w/w^e >> w^p/w^e$

The results indicate an unexpected reversal of order wherein the pearl compounds are lighter than corresponding white compounds, although pearl-homozygotes are darker than white. The rest of the seriation agrees with what one might anticipate. The reversal was so unusual that the experiment was repeated more carefully. In the repetition, honey compounds with eosin, cherry and apricot were omitted, since there was no doubt about their relation to the corresponding compounds of tinged. Honey was now used as a fourth allele with which white, pearl and tinged could be compounded. Care was taken to regulate humidity, temperature (range of 22 to 27° C.), and to standardize the food.

The results of the second experiment are outlined below:

Honey compounds:	age not over 1.5 hours	$w^t/w^h >> w/w^h = w^p/w^h$
	number of flies compared	20      38      39
	average age 5 days	$w^t/w^h > w/w^h = w^p/w^h$
Apricot compounds:	number of flies compared	6      29      26
	age not over 1.5 hours	$w^t/w^a > w/w^a = w^p/w^a$
	number of flies compared	10      35      30
	average age 5 days	$w^t/w^a > w/w^a = w^p/w^a$
	number of flies compared	8      28      27

Cherry compounds:	age not over 2 hours	$w^t/w^{ch} \geq w/w^{ch} > w^p/w^{ch}$
	number of flies compared	14      10      10
	age not over 3 hours	$w^t/w^{ch} = w/w^{ch} > w^p/w^{ch}$
	number of flies compared	19      19      15
	average age 5 days	$w^t/w^{ch} \geq w/w^{ch} > w^p/w^{ch}$
Eosin compounds:	number of flies compared	21      13      16
	age not over 2 hours	$w^t/w^e = w/w^e > w^p/w^e$
	number of flies compared	14      19      31
	average age 5 days	$w^t/w^e = w/w^e > w^p/w^e$
	number of flies compared	11      18      29

Except for a few minor inconsistencies the two sets of data show good agreement. For instance, the initial work gave  $w/w^a \geq w^p/w^a$ , which is not confirmed by the second observation  $w/w^a = w^p/w^a$ . To be sure, there were among the latter some  $w/w^a > w^p/w^a$ , but there were also a few  $w^p/w^a > w/w^a$ , with the greatest number being inseparable. Faced with results neither consistent nor sharp the safest decision seemed to be  $w/w^a = w^p/w^a$ . This inconsistency was also noted in the new honey compounds, which fitted into a series similar to that of the apricot compounds.

About 25 to 50 per cent. of the three-hour-old cherry and five-day-old eosin compounds included such relationships as  $w/w^{ch} > w^t/w^{ch}$  and  $w/w^e > w^t/w^e$ , respectively. But the inverse order also existed in these cases and warranted the insertion of an equality sign. The reason why the white compounds often equal or surpass the color intensity of the tinged compounds of cherry and eosin is probably that, given enough time (five days), the former finally catch up with the latter; that is, the rate of pigment reduction is slower in the white than in the tinged compounds, but the white compounds ultimately reach a state of heavy pigmentation.

The best agreement of data was achieved among the eosin and cherry compounds, as the following comparisons show:

Cherry compounds:	First run, age about 2 hours	$w^t/w^{ch} \geq w/w^{ch} \geq w^p/w^{ch}$
	Second run, age about 2 hours	$w^t/w^{ch} \geq w/w^{ch} > w^p/w^{ch}$
	First run, age about 8 hours	$w^t/w^{ch} \geq w/w^{ch} > w^p/w^{ch}$
	Second run, age about 5 days	$w^t/w^{ch} \geq w/w^{ch} > w^p/w^{ch}$
Eosin compounds:	First run, age about 2 hours	$w^t/w^e \geq w/w^e > w^p/w^e$
	Second run, age about 2 hours	$w^t/w^e = w/w^e > w^p/w^e$
	First run, age about 5 hours	$w^t/w^e = w/w^e > w^p/w^e$
	Second run, age about 5 days	$w^t/w^e = w/w^e > w^p/w^e$

In compound with these two darker alleles, the diluting effect of pearl was always greater than that of white.

A few observations were made of pearl when placed opposite a deficiency, Notch 8. At ages from three hours to five days  $w^p w^p$  was always definitely darker than  $w^p/N8$ . This shows that pearl behaves like other white alleles when opposite a deficiency.

## DISCUSSION AND SUMMARY

The seriation found above in the white and tinged compounds of honey, apricot, cherry and eosin may be regarded as agreeing with the essentials of the usual rate concept. Proceeding from wild type to white, the sequence could be described by assigning to each allele a decreasing rate of reduction on some basic pigment substance. Assuming that two alleles act independently in a compound and that they are the sole variables involved, then the eye color intensity in the compound should be intermediate between what it is in the two homozygotes. If this is true, then several compounds of a single allele, like eosin, with a few other alleles, like tinged and pearl and white, should fit into a regular series corresponding to the order of darkness of these latter alleles. This is apparently what happens when white and tinged are part of the compound, but not when pearl is involved. The pearl and white compounds show an unexpected reversal.

Not all of the four series of compounds show the same degree of reversal. It occurs quite frequently in cherry and eosin compounds and is very marked in the latter. The same can not be said for honey and apricot compounds, although the evidence does permit the negative conclusion that the pearl compounds of these alleles are not (as one might expect them to be) darker than the white. A generalization can be made in which it appears that the darker the original allele used ( $w^e/w^e > w^{ch}/w^{ch} > w^a/w^a > w^h/w^h$ ), the more striking is the white-pearl reversal, thus at about two hours old,  $w/w^e > w^p/w^e$ ,  $w/w^{ch} \cong w^p/w^{ch}$ ,  $w/w^a = w^p/w^a$ ,  $w/w^h = w^p/w^h$ . An interpretation of this unusual reversal of the order of effects of alleles remains to be found.

The experiments were performed as a member of a course in genetics given by L. C. Dunn and A. G. Steinberg. The observations have since been confirmed by Miss Y. Nakayama.

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# INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS

- ABRAMOWITZ, A. A., Pituitary Control of Chromatophores in the Dogfish, 208
- ANDERSON, E., Hindrance to Gene Recombination Imposed by Linkage: An Estimate of Its Total Magnitude, 185
- Aphids, Gamie Female, Order of Differentiation in Relation to Order of Determination in, C. A. LAWSON, 69; Distribution of Intermediate-Winged in the Family and Its Bearing on the Mode of Their Production, A. F. SHULL, 256
- ASMUNDSON, V. S., On the Measurement and Inheritance of Sexual Maturity in Turkeys, 365
- Biology, Human, Some Contributions of the Laboratory Rodents to Our Understanding of, C. C. LITTLE, 127; Use of the Monkey and Ape in Study of, with Special Reference to Primate Affinities, C. G. HARTMAN, 139
- BROWN, F. A., JR., Humoral Control of Crustacean Chromatophores, 247; Coloration and Color Changes of the Gulf-Weed Shrimp, 564
- Cell Theory: Its Past, Present and Future, J. MAYER, 481; Microscopy, L. L. WOODRUFF, 485; Schleiden's Contribution, J. S. KARLING, 517; Predecessors of Schleiden and Schwann, E. G. CONKLIN, 538
- Chimpanzee, Life History and Personality, R. M. YERKES, 97
- Chromatophores, in Relation to Genetic and Specific Distinctions, H. B. GOODRICH, 198; Pituitary Control, in the Dogfish, A. A. ABRAMOWITZ, 208; Humoral Control of Crustacean, F. A. BROWN, JR., 247
- Chromosomal Crepusculum, E. C. JEFFREY and E. J. HAERTL, 560
- Chromosome, Association in *Mesostoma ehrenbergii* (Focke) Schmidt, L. HUSTED, F. F. FERGUSON and M. A. STIREWALT, 180; Shape in *Sciara*, Evolutionary Change in, H. V. CROUSE, 476; Symposium on, Structure: On Coiling, B. R. NEBEL, 289; Physicochemical Nature of the Chromosome and the Gene, C. H. WADDINGTON, 300; Structure of Salivary Gland Chromosome, T. S. PAINTER, 315; as Viewed by a Geneticist, M. DEMEEC, 331
- Chromosomes, Sex, Reinvestigation of the So-called, in *Melandrium* (*Lychnis*) album, H. W. JENSEN, 279; On Coiling in, B. R. NEBEL, 289; Structure of Salivary Gland, T. S. PAINTER, 315; Visible Organization of the Giant Salivary Gland, of Diptera, C. W. METZ, 457
- CLOUDMAN, A. M., C. C. LITTLE, W. S. MURRAY and, Genetics on Non-Epithelial Tumor Formation in Mice, 467
- Color Changes, of *Anolis carolinensis* (Cuvier), F. H. WILSON, 190; Symposium on, in Animals, Their Significance and Activation: Introductory Remarks, G. H. PARKER, 193; Chromatophores in Relation to Genetic and Specific Distinctions, H. B. GOODRICH, 198; Pituitary Control of Chromatophores in the Dogfish, A. A. ABRAMOWITZ, 208; Quantitative Effects of Visual Stimuli upon Pigmentation, F. B. SUMNER, 219; Responses of Melanophores in Isolated Fish Scales, D. C. SMITH, 235; Humoral Control of Crustacean Chromatophores, F. A. BROWN, JR., 247
- Commensalism and Domestication, E. SCHWARZ, 270
- CONKLIN, E. G., Predecessors of Schleiden and Schwann, 538
- CROUSE, H. V., Evolutionary Change in Chromosome Shape in *Sciara*, 476
- CUMLEY, R. W., Precipitin Absorption with *Drosophila* Antigens, 375
- CUNNINGHAM, B., Effect of Temperature upon the Developmental Rate of the Embryo of the Diamond Back Terrapin, 381; M. W. WOODWARD and J. PRIDGEN, Further Studies on Incubation of Turtle Eggs, 285

- DEMEREK, M., Chromosome Structure as Viewed by a Geneticist, 331
- Differential Growth and Evolution in a Subterranean Isopod, M. A. MILLER and E. A. HOY, 347
- Digestion, Change in Gastric, of Kingfishers with Development, H. C. WHITE, 188
- DOBZHANSKY, TH., and K. MATHER, Morphological Differences Between the "Races" of *Drosophila pseudoobscura*, 5
- Drosophila, pseudoobscura*, Morphological Differences Between the "Races," K. MATHER and TH. DOBZHANSKY, 5; *melanogaster*, Failure of Host Genotype to Affect Crossing-Over in an Implanted Ovary in, A. G. STEINBERG and E. C. WHITE, 91; Occurring under Neutron Bombardment, Note of Modifications in the Morphogenesis of, E. V. ENZMANN and C. P. HASKINS, 470; Mass Mutation in the Florida Stock, R. GOLDSCHMIDT, 547; Antigens, Precipitin Absorption with, R. W. CUMLEY, 375; Bar-Eyed, Facet Frequency Distribution in, O. S. MARGOLIS, 472; A New Allele in the Series of, and Its Relationship to Some Other White Alleles, S. GOLDWEBER, 568
- Embryo of the Diamond Back Terrapin, Effect of Temperature upon the Developmental Rate of, B. CUNNINGHAM, 381
- ENZMANN, E. V., and C. P. HASKINS, Note of Modifications in the Morphogenesis of *Drosophila melanogaster* Occurring under Neutron Bombardment, 470
- Euplotes, Mating Types in, R. F. KIMBALL, 451
- Evolution, Building Stones to a Chemistry of, O. RAHN, 26; Genus and Species in Relation to, and to System, H. MILLER, 93
- FERGUSON, F. F., L. HUSTED and M. A. STIREWALT, Chromosome Association in *Mesostoma ehrenbergii* (Focke) Schmidt, 180
- Gene Recombination, Hindrance Imposed by Linkage: An Estimate of Its Total Magnitude, E. ANDERSON, 185
- Genetics of Non-Epithelial Tumor Formation in Mice, C. C. LITTLE, W. S. MURRAY and A. M. CLOUDMAN, 467
- GEISE, A. C., Studies on Conjugation in *Paramecium multimicronucleatum*, 432
- GILMAN, L. C., Mating Types in *Paramecium caudatum*, 445
- GOLDSCHMIDT, R., Mass Mutation in the Florida Stock of *Drosophila melanogaster*, 547
- GOLDWEBER, S., A New Allele in the White Series of *Drosophila* and Its Relationship to Some Other White Alleles, 568
- GOODRICH, H. B., Chromatophores in Relation to Genetic and Specific Distinctions, 198
- GREENSHIELDS, F., Whiting's Hypothesis and *Pteromalus*: A Critique of Dozorceva's (1936) Study, 89
- Growth Constants, Relative, Dimensions and Interrelationship of, H. LUMER, 339
- Gulf-Weed Shrimp, Coloration and Color Changes, F. A. BROWN, JR., 564
- HAERTL, E. J., and E. C. JEFFREY, Chromosomal Crepusculum, 560
- HARTMAN, C. G., Use of the Monkey and Ape in the Study of Human Biology, with Special Reference to Primate Affinities, 139
- HASKINS, C. P., and E. V. ENZMANN, Note of Modifications in the Morphogenesis of *Drosophila melanogaster* Occurring under Neutron Bombardment, 470
- Higher Animals, Contributions of, to Understanding of Human Biology: Experimental Animal from Naturalists' Point of View, G. K. NOBLE, 113; Laboratory Rodents, C. C. LITTLE, 127; Monkey and Ape, C. G. HARTMAN, 139
- HOY, E. A., and M. A. MILLER, Differential Growth and Evolution in a Subterranean Isopod, 347
- HUSTED, L., F. F. FERGUSON and M. A. STIREWALT, Chromosome Association in *Mesostoma ehrenbergii* (Focke) Schmidt, 180
- Insects, Longevity of, During Complete Inanition, N. S. R. MALUF, 280

- JEFFREY, E. C., and E. J. HAERTL, Chromosomal Crepusculum, 560
- Jellyfish, Freshwater, Records Since 1932, W. L. SCHMITT, 83
- JENNINGS, H. S., Introduction to the Symposium on Mating Types and Their Interactions in the Ciliate Infusoria, 385; *Paramecium bursaria*; Mating Types and Groups, Mating Behavior, Self-Sterility; Their Development and Inheritance, 414
- JENSEN, H. W., A Reinvestigation of the So-Called Sex Chromosomes in *Melandrium (Lychnis) album*, 279
- KARLING, J. S., Schleiden's Contribution to the Cell Theory, 517
- KIMBALL, R. F., Mating Types in Euplotes, 451
- LAWSON, C. A., Order of Differentiation in Relation to Order of Determination in Gametic Female Aphids, 69
- LITTLE, C. C., Some Contributions of the Laboratory Rodents to our Understanding of Human Biology, 127; W. S. MURRAY and A. M. CLOUDMAN, Genetics of Non-Epithelial Tumor Formation in Mice, 467
- LUMER, H., Dimensions and Interrelationship of the Relative Growth Constants, 339
- MALUF, N. S. R., Longevity of Insects During Complete Inanition, 280
- MARGOLIS, O. S., Facet Frequency Distribution in Bar-Eyed *Drosophila*, 472
- MATHER, K., and TH. DOBZHANSKY, Morphological Differences Between the "Races" of *Drosophila pseudoobscura*, 5
- Mating Types and Their Interactions in the Ciliate Infusoria: Introduction, H. S. JENNINGS, 385; *Paramecium aurelia*, T. M. SONNEBORN, 390; *Paramecium bursaria*, H. S. JENNINGS, 414; *Paramecium multimicronucleatum*, A. C. GIESE, 432; *Paramecium caudatum*, L. C. GILMAN, 445; Euplotes, R. F. KIMBALL, 451
- MAYER, J., Cell Theory, Its Past, Present and Future, 481
- MAYR, E., Sex Ratio in Wild Birds, 156
- Melanophores, Responses of, in Isolated Fish Scales, D. C. SMITH, 235
- METZ, C. W., Visible Organization of the Giant Salivary Gland Chromosomes of Diptera, 457
- Microscopy Before the Nineteenth Century, L. L. WOODRUFF, 485
- MILLER, H., Genus and Species in Relation to Evolution and to System, 93
- MILLER, M. A., and E. A. HOY, Differential Growth and Evolution in a Subterranean Isopod, 347
- MURRAY, W. S., C. C. LITTLE and A. M. CLOUDMAN, Genetics of Non-Epithelial Tumor Formation in Mice, 467
- Naturalists, American Society of, 97-155
- NEBEL, B. R., On Coiling in Chromosomes, 289
- NOBLE, G. K., Experimental Animal from the Naturalist's Point of View, 113
- PAINTER, T. S., Structure of Salivary Gland Chromosomes, 315
- Paramecium aurelia*: Mating Types and Groups; Lethal Interactions: Determination and Inheritance, T. M. SONNEBORN, 390; *bursaria*: Mating Types and Groups, Mating Behavior, Self-Sterility; Their Development and Inheritance, H. S. JENNINGS, 414; *multimicronucleatum*, Studies on Conjugation in, A. C. GIESE, 432; *caudatum*, Mating Types in, L. C. GILMAN, 445
- PARKER, G. H., Introductory Remarks, Symposium on Color Changes in Animals, 193
- Pigmentation, Quantitative Effects of Visual Stimuli upon, F. B. SUMNER, 219
- Predecessors of Schleiden and Schwann, E. G. CONKLIN, 538
- PRIDGEN, J., B. CUNNINGHAM and M. W. WOODWARD, Further Studies on Incubation of Turtle Eggs, 285
- RAHN, O., Building Stones to a Chemistry of Evolution, 26
- SCHMITT, W. L., Freshwater Jellyfish Records Since 1932, 83
- SCHWARZ, E., Commensalism and Domestication, 270



- Sex Ratios, and Twin Producing Kindreds, W. E. SOUTHWICK, 44; in Wild Birds, E. MAYR, 156
- Sexual Maturity in Turkeys, On the Measurement and Inheritance of, V. S. ASMUNDSON, 365
- SHULL, A. F., Distribution of Intermediate-Winged Aphids in the Family and Its Bearing on the Mode of Their Production, 256
- SMITH, D. C., Responses of Melanophores in Isolated Fish Scales, 235
- Somatic Crossing-Over and Somatic Translocation, C. STERN, 95
- SONNEBORN, T. M., *Paramecium aurelia*; Mating Types and Groups; Lethal Interactions; Determination and Inheritance, 390
- SOUTHWICK, W. E., Sex Ratios and Twin Producing Kindreds, 44
- STEINBERG, A. G., and E. C. WHITE, Failure of Host Genotype to Affect Crossing-Over in an Implanted Ovary in *Drosophila melanogaster*, 91
- STERN, C., Somatic Crossing-Over and Somatic Translocations, 95
- STIREWALT, M. A., L. HUSTED and F. F. FERGUSON, Chromosome Association in *Mesostoma ehrenbergii* (Focke) Schmidt, 180
- SUMNER, F. B., Quantitative Effects of Visual Stimuli upon Pigmentation, 219
- Turtle Eggs, Further Studies on Incubation of, B. CUNNINGHAM, M. W. WOODWARD and J. PRIDGEN, 285
- WADDINGTON, C. H., Physicochemical Nature of the Chromosome and the Gene, 300
- WHITE, E. C., and A. G. STEINBERG, Failure of Host Genotype to Affect Crossing-Over in an Implanted Ovary in *Drosophila melanogaster*, 91
- WHITE, H. C., Change in Gastric Digestion of Kingfishers with Development, 188
- Whiting's Hypothesis and *Pteromalus*: A Critique of Dozorzeva's (1936) Study, F. GREENSHIELDS, 89
- WILSON, F. H., Preliminary Experiments on Color Changes of *Anolis carolinensis* (Cuvier), 190
- WOODRUFF, L. L., Microscopy Before the Nineteenth Century, 485
- WOODWARD, M. W., B. CUNNINGHAM and J. PRIDGEN, Further Studies on Incubation of Turtle Eggs, 285
- YERKES, R. M., Life History and Personality of Chimpanzee, 97
- Zoologists, American Society of, 193-255; 289-338; 385-456





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